Effect of Erythropoietin on Activity of Plasma Proteolytic Systems during Experimental Renal Failure

M. V. Osikov and T. A. Grigoriev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 153, No. 1, pp. 27-30, January, 2012
Original article submitted October 28, 2010

Activity of plasma proteolytic systems (fibrin formation, fibrinolysis, and anticoagulant system) and the possibility for correction of changes in these systems with erythropoietin were studied in experiments on outbred albino rats with experimental renal failure. Renal failure was induced by a single subcutaneous injection of mercury chloride (II). The parameters were estimated on day 5 postinjection. Erythropoietin in a single dose of 5000 U/kg was administered on day 4. Renal failure was accompanied by activation of fibrin formation (with factors of the common and intrinsic pathways of blood coagulation), increase in antithrombin activity, and inhibition of the fibrinolytic system. Treatment with erythropoietin led to partial recovery of fibrin formation and fibrinolysis. Under analytical *in vitro* conditions, 30-min incubation of whole blood from healthy donors with erythropoietin in doses of 1.9-30.0 U/liter was followed by a dose-dependent inhibition of the fibrin formation system and activation of fibrinolysis.

Key Words: *erythropoietin; renal failure; plasma proteolytic systems; blood coagulation; fibrinolysis*

Dysregulation of plasma proteolytic systems of fibrin formation and fibrinolysis and the anticoagulant system can serve as a key pathogenetic factor for thrombohemorrhagic complications in renal failure of different genesis [4,13]. Erythropoietin (EPO) is a glycoprotein (molecular weight 30.4 kDa) responsible for proliferation, differentiation, and inhibition of apoptosis in glycoprotein-sensitivebone marrow erythroid lineage cells. Much attention is now focused on non-hemopoietic effects of EPO. Previous studies showed that EPO affects vascular myocytes, cardiomyocytes, endotheliocytes, neurons, retinal ganglion cells, lung epithelium, and other cells [2,14]. EPO improves activity of the erythron system in renal failure. This compound has a corrective effect on dysfunction of the nervous and cardiovascular system. Little is known about the effects of EPO on plasma proteolytic systems, whose components regulate functional activity of blood cells and endotheliocytes and modulate the cell-humoral interactions.

Here we studied the effect of EPO on the plasma proteolytic systems under conditions of experimental renal failure.

MATERIALS AND METHODS

Experiments were performed on 60 male outbred albino rats weighing 200-220 g. The animals were randomized into the following four groups: group 1, intact rats; group 2, rats with acute renal failure (ARF); group 3, rats with ARF receiving EPO; and group 4, intact rats receiving EPO. ARF was induced by subcutaneous injection of mercury chloride (II) in a single dose of 5 mg/kg (into the interscapular region). Control animals received an equivalent volume of physiological saline. ARF was verified by a morphological and biochemical study. The concentrations of urea and creatinine in blood plasma were measured with Bio-La-Test kits (PLIVA-Lachema). Morphological signs

Department of Pathophysiology, Chelyabinsk State Medical Academy, Ministry of Health and Social Development of the Russian Federation. ***Address for correspondence:*** mvo2003@list.ru. M. V. Osikov

0007-4888/12/1531-0021

of the disease included epithelial cell necrosis in the nephron tubules.

EPO (Eral'fon, INN epoetin alpha, Soteks pharmaceutical company) in a single dose of 5000 U/kg was injected intraperitoneally to rats of groups 3 and 4. Group 3 animals received EPO on day 4 after ARF modeling. The rats were examined on day 5 (groups 2 and 3) or 2 after ARF induction (group 4). The blood was sampled from diethyl ether-anesthetized rats by puncture of the left ventricle. Blood samples were stabilized with 3.8% sodium citrate in the 1:9 ratio (w/v). Platelet-rich plasma and platelet-poor plasma were obtained by centrifugation at 150*g* and 1200*g*, respectively. For *in vitro* study, whole blood from 24 healthy donors was used (Chelyabinsk regional hemotransfusion station). EPO was administered in doses of 30, 15, 7.5, 3.75, and 1.88 U/liter, which corresponds to 200, 100, 50, 25, and 12.5% EPO in blood serum under normal conditions. Activity of the coagulation cascade was estimated from the thrombin time, activated partial thromboplastin time (APTT), and plasma fibrinogen concentration [1]. Functions of the anticoagulant system were evaluated from antithrombin activity. Plasma fibrinolytic activity was determined from the time of XIIa-kallikrein-dependent fibrinolysis. Experiments were performed on an APG2-02 coagulometer (Tekhnomedika) with Tekhnologiya-standart reagents.

The results were analyzed by Statistica 6.0 software [6].

RESULTS

ARF was accompanied by activation of the coagulation cascade, increase in antithrombin activity, and acceleration of fibrinolysis (Table 1). Activation of the coagulation cascade is mainly associated with factors of the common and intrinsic pathways. This conclusion was made from the decrease in the prothrombin time and APTT by 19 and 43%, respectively (in comparison with intact animals). The decrease in the thrombin time reflects acceleration of the final stage of blood coagulation. At first glance, hyperfibrinogenemia seems to contradict the observed changes. This state probably develops during the acute phase response to renal tissue injury, since fibrinogen is a positive acute-phase reactant [3].

Activation of the intrinsic pathway of blood coagulation in renal failure can be caused by contact with polyanion surfaces (*e.g.*, uremic toxins). Variations in antithrombin activity and fibrinolytic system are probably associated with hypercoagulation during renal failure. The increase in plasma antithrombin activity probably results from inactivation of coagulation factors. Inhibition of the fibrinolytic system is usually related to plasminogen deficiency [1]. Streptokinase is extensively used to evaluate the role of various components (factor XII, prekallikrein, high-molecular weight kininogen, plasminogen, and their inhibitors) in these processes. Streptokinase does not normalize the process of lysis under conditions of plasminogen deficiency. This possibility was excluded, because rat plasminogen is practically insensitive to streptokinase [7]. Deceleration of XIIa-dependent euglobulin lysis due to consumption of fibrinolytic components during hypercoagulation in renal failure is confirmed by published data on accumulation of fibrin-monomer and D-dimer in blood plasma [9]. Inhibition of fibrinolysis is related not only to consumption of the main components, but also to increased activity of plasminogen activator inhibitors PAI-1 and PAI-2 during renal failure [8].

Administration of EPO to ARF animals was followed by a decrease in activity of the fibrin formation system (Table 1). Thrombin time, prothrombin time, and APTT returned to normal after EPO treatment. The concentration of fibrinogen in EPO-treated animals did not differ from the control. The effect of EPO on coagulation is not mediated by changes in blood antithrombin activity. There are contradic-

TABLE 1. Effect of EPO on Activity of the Plasma Proteolytic System in Rats (*M*±*m*)

Parameter	Group 1 (intact, *n*=19)	Group 2 (ARF, *n*=18)	Group 3 (ARF+EPO, *n*=13)	Group 4 (intact+EPO, *n*=10)
TT, sec	59.58±9.71	24.55±3.57*	64.32±10.59^{+}	52.93±6.26
APTT, sec	32.13±3.98	18.21±1.00*	24.98±2.66^{+}	27.95±4.09
PTT, sec	27.48±2.16	22.25±0.10*	29.85±2.45^{+}	39.37±6.76
Fibrinogen, g/liter	1.84±0.17	2.83±0.12*	2.81±0.38*	2.09±0.24
PFA, min	11.41±1.07	6.58±0.69*	14.07±2.69*$^{+}$	12.40±0.76
AAT, sec	104.96±8.02	131.09±3.92*	129.08±10.39	98.66±10.49

Note. Here and in Table 2: TT, thrombin time; PTT, prothrombin time; AAT, antithrombin activity; PFA, plasma fibrinolytic activity. $p<0.05$: *compared to group 1; $^{+}$compared to group 2 (Mann-Whitney test).

TABLE 2. Effect of EPO on Activity of the Plasma Proteolytic System under *In Vitro* Conditions (n=8, $M\pm m$)

Parameter	Blood+physiological saline (control)	Blood+EPO, 1.88 U/liter	Blood+EPO, 3.75 U/liter	Blood+EPO, 7.5 U/liter	Blood+EPO, 15 U/liter	Blood+EPO, 30 U/liter
TT, sec	14.65±0.49	15.73±0.35*	16.05±0.65*	18.03±1.05*	16.60±0.85*	17.10±0.70*
APTT, sec	33.53±0.68	33.30±1.36	34.33±1.66	40.18±4.69	33.20±1.82	39.40±1.44*
PTT, sec	15.30±0.59	15.63±0.66*	17.53±1.05*	18.08±0.99*	16.90±0.78*	16.73±0.68*
Fibrinogen, g/liter	3.16±0.21	3.08±0.28	2.83±0.22	2.99±0.24	3.03±0.23	2.99±0.25
PFA, min	5.58±0.17	4.90±0.20*	5.05±0.16*	4.90±0.55	5.15±0.06	4.98±0.21*
AAT, sec	105.30±2.63	111.43±4.94	100.85±5.59	109.88±5.92	102.80±5.83	109.57±2.28

Note. *p<0.05 in comparison with the control (Wilcoxon test).

tory data on the effect of EPO on plasma proteolytic systems during renal failure. Some authors believe that EPO has a normalizing effect on blood coagulation activity and fibrinolysis in patients with chronic renal failure, while others report the ineffectiveness of EPO [10,11]. This discrepancy can be related to the necessity of anticoagulant treatment in renal failure patients. The effects of EPO on activity of plasma proteolytic systems during renal failure are probably associated with direct nephroprotective action of this glycoprotein. EPO receptors were identified not only on erythroid cells, but also in the nervous tissue, ovaries and testicles, uterus, myocytes, endotheliocytes, and nephron epithelium [2,14]. These data suggest that EPO has several functions (distinct from hemopoietic activity). EPO inhibits apoptosis, stimulates mitotic processes in tubular cells, and contributes to a rapid recovery of renal functions in rats with experimental renal ischemia [12,15]. It cannot be excluded that the effects of EPO are related to detoxification properties of this compound. Our previous studies showed that EPO significantly decreases the concentration of serum creatinine during chronic renal failure [5].

EPO was administered to intact animals to evaluate whether or not the effects of this compound on plasma proteolytic systems depend on the pathological state. The effects of EPO on the fibrin formation system, duration of XIIa-kallikrein-dependent fibrinolysis, and antithrombin activity were statistically insignificant. Under analytical conditions *in vitro*, EPO had a modulatory effect on coagulation hemostasis after 30-min incubation with whole blood from healthy donors (Table 2). EPO in doses of 30, 15, 7.5, 3.75, and 1.88 U/liter (corresponding to 200, 100, 50, 25, and 12.5% EPO in blood serum under normal conditions) produced the hypocoagulation effect, which involved the common pathway factors. The *in vitro* effects of EPO are probably related to a short-term experiment and direct contact of this glycoprotein with blood proteins (components of plasma proteolytic systems). A correlation analysis showed that EPO has a direct dose-dependent effect on the fibrin formation system. The Spearman correlation coefficients for the thrombin time and prothrombin time were R=0.37 (p<0.05) and R=0.29 (p<0.05), respectively. A one-way analysis of variance showed that EPO modulates activity of the fibrin formation system (p<0.05). The effect of EPO on the thrombin time was not less than 21.65%, but not more than 72.28% of the total effect of all factors. The effect of EPO on the prothrombin time was not less than 19.94%, but not more than 64.32% of the total effect of all factors.

Our results indicate that administration of EPO in a dose of 5000 U/kg is followed by partial recovery of the fibrin formation and fibrinolysis systems during experimental renal failure. *In vitro* study showed that EPO has a direct dose-dependent effect on the plasma systems of fibrin formation and fibrinolysis.

REFERENCES

1. Z. S. Barkagan, *Diagnostics and Controlled Therapy of Hemostatic Disorders* [in Russian], Moscow (2001).
2. Yu. M. Zakharov, *Klin. Nefrol*., No. 1, 16-21 (2009).
3. P. G. Nazarov, *Reactants of the Acute Inflammatory Phase* [in Russian], St. Petersburg (2001).
4. M. V. Osikov, *Byull. Eksp. Biol. Med*., **148**, No. 7, 27-30 (2009).
5. M. V. Osikov, *Vestn. Yuzhno-Ural'sk. Gos. Un-ta. Ser. Obrazovanie, Zdravookhranenie, Fizicheskaya Kul'tura*, No. 19, Vyp. 23, 92-96 (2010).
6. O. Yu. Rebrova, *Statistical Analysis of Medical Data. Use of STATISTICA Software* [in Russian], Moscow (2006).
7. E. E. Shimonaeva, *Vopr. Med. Khimii*, **34**, No. 3, 28-29 (1988).
8. M. Bronisz, D. Rosc, A. Bronisz, *et al*., *Ren. Fail*., **26**, No. 3, 223-229 (2004).
9. J. Malyszko, J. S. Malyszko, D. Pawlak, *et al*., *Thromb. Res*., **83**, No. 5, 351-361 (1996).
10. K. Pawlak, D. Pawlak, and M. Mysliwiec, *Thromb. Res.*, **120**, No. 6, 797-803 (2007).

11. K. Pawlak, D. Pawlak, and M. Mysliwiec, *Thromb. Res.*, **121**, No. 6, 787-791 (2008).
12. E. J. Sharples and M. M. Yagoob, *Nephron Exp. Nephrol.*, **104**, No. 3, e83-e88 (2006).
13. I. Stefanidis, D. Frank, and N. Maurin, *Ren. Fail.*, **20**, No. 1, 147-155 (1998).
14. E. Taoufik, E. Petit, D. Divoux, *et al.*, *Proc. Natl. Acad. Sci. USA*, **105**, No. 16, 6185-6190 (2008).
15. D. A. Vesey, C. Cheung, B. Pat, *et al.*, *Nephrol. Dial. Transplant.*, **19**, No. 2, 348-355 (2004).