Effect of Recombinant Human Erythropoietin on the Rate of Na^+,H^+-Exchange in Erythrocytes from Patients with Chronic Renal Failure on Hemodialysis

A. A. Kubatiev, I. A. Rud'ko, and V. M. Ermolenko

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The study explores the effect of recombinant human erythropoietin and the rate of Na^+,H^+-exchange in erythrocytes from patients with chronic renal failure undergoing hemodialysis. The rate of Na^+,H^+-exchange in erythrocytes from patients was higher than in the control and remained unchanged after 24 months of treatment with erythropoietin. Therapy with recombinant human erythropoietin does not normalize the Na^+,H^+-exchange mechanism. It is concluded that factors underlying disturbances of ion transport in erythrocytes from uremic patients cannot be corrected with erythropoietin.

Key Words: *erythropoietin; Na^+,H^+-exchange; erythrocyte; hemodialysis*

Anemia in patients with end-stage chronic renal failure (CRF) undergoing hemodialysis is caused by relative erythropoietin (EP) deficiency and enhanced hemolysis. Mechanism of hemolysis in patients with CRF is poorly understood. Previous studies demonstrated a reduced osmotic resistance of erythrocytes from patients with CRF [4]. Na^+,H^+-exchange plays an essential role in the maintenance of the erythrocyte shape and osmotic regulation [7]. Activation of Na^+,H^+-exchange leads to elevation of intracellular pH and Na^+ entry into erythrocytes, which is crucial for regulation of the erythrocyte volume [6].

Activators of protein kinase C stimulate Na^+,H^+-exchange in human erythrocytes [11]. The binding of recombinant human EP (rhEP), a preparation for the treatment of anemia in hemodialysis patients, to receptors on EP-dependent target cells leads to simultaneous or successive activation of tyrosine and serotonin kinases. The latter enzyme can be an isoform of protein kinase C [12].

We studied the effect of rhEP on the rate of Na^+,H^+-exchange in erythrocytes from CRF patients on hemodialysis.

MATERIALS AND METHODS

Eight hemodialysis patients with end-stage CRF (22-61 years old) were examined. All patients had severe anemia (hemoglobin 79.5±5.5 g/liter, hematocrit 23.2±1.8%) Hemodialysis was performed 3 times per week on a DIP-02-02 dialysers (1 m^2 exchange area) with a cuprofane membrane.

The patients were subcutaneously injected with rhEP (20 U/kg, Boehringer Mannheim) 3 times per week 10 min before hemodialysis. If 1-month treatment with this dose was ineffective, the dose was doubled. The desired level of hematocrit was 31-35%. Blood samples from 10 healthy volunteers served as the control.

Sodium citrate blood (3.8%) drawn from the ulnar vein before hemodialysis was centrifuged for 10

Department of General Pathology and Pathological Physiology, Department of Nephrology, Russian Academy of Postgraduate Education, Moscow

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min at 1000*g* and 2-4°C. Plasma and leukocyte coat was removed, while the erythrocyte sediment was twice washed with physiological saline containing 5 mM sodium phosphate buffer, pH 7.4.

The rate of Na^+,H^+-exchange (μmol H^+/liter cells/min) was measured as an amiloride-sensitive component of proton efflux under conditions of electrochemical proton gradient with extra- and intracellular pH of 6.45 and 8.00, respectively (ΔμH-induced Na^+,H^+-exchange). Detailed procedure and calculation are described elsewhere [1]. Proton efflux was recorded in an F-70 pH-meter with a 39830 combined electrode (Beckman). All reagents were from Serva and Sigma.

The data were processed statistically using the Student's *t* test.

RESULTS

The rate of Na^+,H^+-exchange in uremic erythrocytes was higher than in the control ($p<0.05$). The highest rate of Na^+,H^+-exchange (from 109.1 to 207.3 μmol H^+/liter cells/min) was observed in erythrocytes from patients with arterial hypertension.

Three months after the start of rhEP therapy we observed a tendency toward an inhibition of Na^+,H^+-exchange in erythrocytes from patients with CRF in comparison with the initial values; however, after 6 and 24 months of rhEP therapy, the rate of Na^+,H^+-exchange did not differ from the correspondent values before treatment. After 24 months, the rate of Na^+,H^+-exchange in erythrocytes from patients remained higher than in the control (Fig. 1).

Rheological properties of erythrocytes depend on the content of hemoglobin, its volume and conformation, which are maintained by ion transporting systems, primarily, Na^+,H^+-antiporter. Our experiments demonstrated activation of Na^+,H^+-exchange in erythrocytes from hemodialysis patients with CRF. This activation may be caused by various factors elevating cytoplasmic calcium and by a decrease in intracellular pH of erythrocytes.

For instance, the above-mentioned shifts can be caused by parathyroid hormone (PTH). Blood concentration of PTH in uremic patients is increased. Effect of PTH on erythrocytes is mediated through cytoplasmic Ca^{2+}. *In vitro* activation of Ca^{2+}-ATPase induced with PTH in erythrocytes from adult and newborn rabbits is higher in young erythrocytes than in mature cells [9]. Uremic serum and blood ultrafiltrate from these patients exert similar effects on young erythrocytes [9].

However, it cannot be excluded that activation of Na^+,H^+-exchange in erythrocytes from patients with CRF is due to the presence of large number of young erythrocytes in peripheral blood [6]. Previous studies have shown enhanced Na^+,H^+-exchange in young erythrocytes followed by its decrease upon maturation [2].

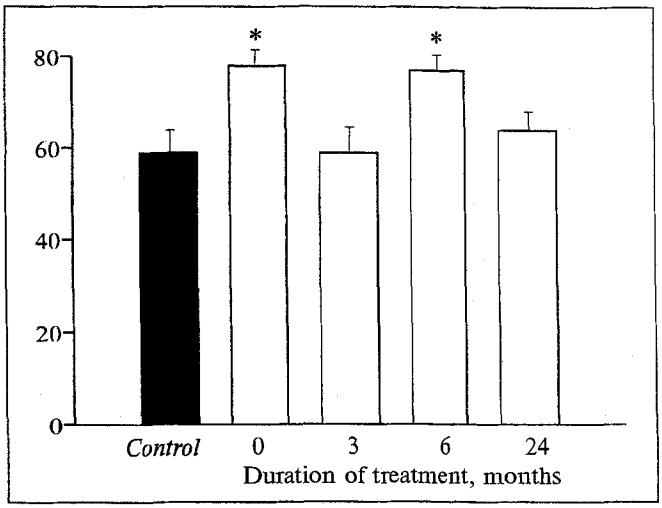

Fig. 1. Rate of Na^+,H^+-exchange (μmol H^+/liter cells/min) in erythrocytes of uremic patients treated with recombinant human erythropoietin. *$p<0.05$ compared with the control.

Recombinant human EP stimulates proliferation and differentiation of the erythropoiesis precursors, erythroblasts and reticulocytes. The binding of rhEP to specific receptors of erythroid cells is accompanied by elevation of cytoplasmic calcium in the bone marrow cells, which is crucial for their differentiation and maturation [3]. Treatment with rhEP modulates rheological properties of erythrocytes: for instance, it reduces elastic modules in the erythrocyte plasma membrane [8]. Previous studies have shown enhanced deformability and significantly increased the volume of erythrocytes in CRF patients treated with rhEP for 5 months [13].

Reduced rate of Na^+,H^+-exchange in uremic erythrocytes observed 3 months after the start of rhEP therapy in comparison with corresponding values before treatment is probably due to reticulocyte maturation induced by the preparation. This assumption is confirmed by low activity of aminotransferase in erythrocytes from uremic patients against the background of rhEP treatment. This attests to an increased number of mature cells, since aminotransferase activity in mature erythrocytes is lower than in young cells.

On the other hand, reduction in the rate of Na^+,H^+-exchange in erythrocytes from patients with CRF 3 months after the start of rhEP therapy may result from an increase in the erythrocyte volume, which was observed in patients [13] and animals [10] against the background of rhEP treatment.

A two-year therapy with rhEP did not normalize the Na^+,H^+-exchange in erythrocytes of CRF pa-

tients. This is probably due to the fact that Na^+,H^+-exchange is a secondary active transport maintained by Na^+ gradient created by Na^+,K^+-APTase. It was shown that Na^+ transport in erythrocytes from uremic patients treated with rhEP for 3 months remained unchanged. *In vitro* incubation of erythrocytes from CRF patients and healthy individuals in the presence of rhEP did not affect Na^+ transport.

Regardless the mechanisms activating Na^+,H^+-exchange in erythrocytes from patients with CRF, our findings suggest that rhEP therapy does not normalize Na^+,H^+-exchange. Disturbances in ion transport in erythrocytes of uremic patients are apparently caused by factors that cannot be corrected by rhEP therapy.

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