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Review Article

The Role of Erythropoietin in the Anemia of Chronic Renal Failure

N. Kevin Krane, MD*

The major factors responsible for the anemia of chronic renal failure are decreased erythropoietin (Ep) production, the presence of inhibitors of erythropoiesis, blood loss, and hemolysis. Ep, which is produced in the kidney, probably exerts its effect on the colony-forming units of the erythrocyte. Ep levels fall with worsening renal function (creatinine clearances in the range of 2-40 ml/min),

but they rise to their highest levels in the immediate predialysis period, probably due to severe ischemia of both renal and extrarenal production sites. When patients are begun on hemodialysis, Ep levels fall, and the hematocrit rises. Erythropoietin therapy in the management of anemia of chronic renal failure is potentially a practical application of experimental studies.

Anemia, an almost invariable feature of chronic renal failure, usually develops when creatinine levels are greater than 3.5 mg/dl, or creatinine clearance is less than 40 ml/min/1.73m² (1). Except in many patients who have polycystic kidney disease, the anemia worsens as renal function deteriorates, and end-stage renal failure is reached. After this has occurred, the degree of anemia remains relatively constant, although it may actually improve when the patient is placed on hemodialysis.

In general, anemia is better tolerated in chronic renal failure than in other chronic illnesses. This tolerance has been attributed to acidemia and to elevated 2,3 DPG levels, which lower hemoglobin-oxygen affinity and facilitate release of oxygen to the tissues (2,3). Symptoms usually occur in those patients with underlying pulmonary disease or with vascular insufficiency, which decreases cardiac or cerebral blood flow.

The anemia of chronic renal failure is a normochromic, normocytic anemia characterized by a decreased reticulocyte index, the appearance of burr cells in the peripheral blood smear, and a "normal" bone marrow morphology. The response of the marrow is actually impaired because erythropoiesis in individuals with a similar degree of anemia is much more active.

The anemia of renal failure has many causes, and several factors contribute to its severity. The major causes are: 1) decreased erythropoietin (Ep) production; 2) presence of inhibitors of erythropoiesis; 3) blood loss; and 4) hemolysis.

Inhibitors of Erythropoiesis

Bone marrow failure, due in part to decreased Ep production, may also be caused by the presence of inhibitors of erythropoiesis in uremic serum. Serum from patients with uremia is capable of inhibiting the rate of erythropoiesis by both human and animal erythroblasts in culture. The inhibitors do not inactivate or interfere with the action of Ep, but, as shown by experiments with cells in culture, they inhibit erythroblast protein synthesis (4). To demonstrate the importance of these inhibitors, Wallner and Vautrin (5) compared the ability of serum from uremic patients and controls to inhibit heme synthesis by rabbit marrow cultures and erythroid colony formation by mouse marrow culture. As renal function worsened and hematocrit fell, serum inhibitor levels rose, as measured by the in vitro systems. Conversely, inhibitor concentrations fell in patients whose hematocrit rose after beginning hemodialysis. Apparently, with worsening renal failure, concentration of a dialyzable inhibitor increases, contributing to marrow suppression.

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Ohno, et al observed that sera from undialyzed anemic, uremic patients significantly inhibited erythroid colony-forming units in rabbits marrows, with reduction in the erythroid precursors or burst-forming units (BFU) (6). After dialysis, the inhibitors of colony-forming units-erythroid (CFU-E) were markedly reduced in the serum of three of four patients. The BFU were not measured. Whether a single inhibitor of erythropoiesis is capable of inhibiting both types of erythroid colony-forming cells (CFU-E and BFU-E) or whether two different specific inhibitors are present was not established.

Blood Loss in Chronic Renal Failure

Excessive blood loss may be due to: 1) gastrointestinal mucosal bleeding due to platelet defects; 2) phlebotomy for laboratory studies; 3) surgical procedures; or 4) blood loss during dialysis. Using technetium-labeled red blood cells and six different dialyzers, Ireland, et al (7) concluded that the annual whole blood loss from all causes was 5.3L, with 1.606 ml from gastrointestinal loss, 540 ml from blood sampling, and a minimum of 3,180 ml from dialysis procedures. During dialysis, blood may be trapped, slowing blood flow and leading to the development of thrombus on the dialysis membrane.

Hemolysis

Average red cell survival determined by ⁵¹chromium tagging is reported to be 63 days in uremic patients compared to 98 days in a control group (8). The shortened red cell survival has been confirmed by other techniques including a bilirubin turnover method (9). Several factors responsible for this problem include impaired erythrocyte pentose phosphate enzymes in uremia, and microangiopathic changes in some patients that permit intravascular trauma to the red cells. Signs of hemolysis may be evident in these patients.

The causes of hemolysis in patients with chronic renal failure (10) include:

1. Erythrocyte sodium pump inhibition by uremic plasma
2. Microangiopathy
3. Decreased intraerythrocytic pentose phosphate shunt activity due to: a) G6PD deficiency; b) chloramine in tapwater used in hemodialysis; c) drugs with oxidizing potential
4. Red blood cell rigidity due to hypophosphatemia
5. Decreased filterability of red blood cells
6. Hypersplenism

7. Thermal injury
8. Increased serum concentration of copper, aluminum, or zinc

Erythropoietin

Impaired ability of the diseased kidney to produce Ep is one factor contributing to bone marrow failure in chronic renal failure. It has been postulated that the kidney produces an enzyme that reacts with a plasma substrate to produce Ep. However, active Ep can be found in the perfusate after perfusion of isolated kidneys with a serum free medium. Biologically active Ep can also be extracted from bovine kidneys homogenized in a neutrally buffered solution.

The actual site of Ep production has not been identified. Fluorescein-labeled globulins against crude Ep localize to the glomeruli; however, because other antibodies may localize there, the glomeruli have not proved to be the site of Ep production. The juxtaglomerular apparatus (JGA) has been proposed as the renal source for Ep because some patients with Bartter's syndrome, who have hyperplasia of the JGA, have erythrocytosis and elevated plasma Ep levels. Finally, the renal medulla has been considered as a possible site because Ep production increases when intramedullary pressure rises. The liver produces Ep during early development, in states of hypoxia, or in adults after nephrectomy. In some patients with renal failure, the liver may contribute significantly to Ep production. With liver damage, e.g., viral or toxic hepatitis, anemia of chronic renal failure may temporarily improve, presumably from production of erythropoietin by regenerating liver cells (11). As will be discussed later, extrarenal sites of Ep production may be important in the response to severe tissue ischemia during renal failure.

Ep production depends on the plasma pO₂, hemoglobin oxygen saturation, hemoglobin concentration, blood flow, erythrocyte 2,3 DPG levels, and the basal metabolic rate (10).

In vitro, Ep induces the earliest erythroid precursors BFU-E, but it has little effect on BFU-E in vivo. The colony-forming units, presumably derived from the BFU, are very sensitive to Ep both in vivo and in vitro. The CFU-E are therefore felt to be the primary site at which Ep exerts its erythropoietic effect. Whether Ep can affect the multipotential hematopoietic stem cell (CFU-S) is uncertain.

Erythropoietin is a glycoprotein that can be assayed in either the plasma or urine. However, the urinary Ep assay

is not valid in patients with renal failure because little is known about Ep excretion. Assays for plasma Ep have been slow to develop because it is difficult to obtain sufficient quantities of purified bioactive Ep from kidney extracts. For this reason, animal cell culture bioassays and a hemagglutination inhibition assay have been employed. Recently, purification of human Ep from the urine of patients with aplastic anemia has provided small quantities of highly bioactive Ep (70,000 U/mg) (12).

In 1981, Zaroulis, et al evaluated Ep levels in polycythemia vera, aplastic anemia, and chronic renal failure (13). Their radioimmunoassay (RIA) for Ep used highly purified radio-iodinated Ep as tracer, while antisera were generated in rabbits using less pure Ep (62 U/mg) as antigen. Ep levels were low in patients with polycythemia vera and high in those with aplastic anemia, indicating that the assay was sufficiently sensitive and specific. They evaluated 19 normal individuals, 9 patients with severe aplastic anemia, 3 with untreated polycythemia vera, and 11 chronic hemodialysis patients.

In normal patients, Ep levels ranged from 18-81 with a mean of 29 mU/ml. In patients with severe aplastic anemia, levels of Ep were markedly elevated (mean value of 3,487 mU/ml), while in the three patients with polycythemia, the mean level was 18 mU/ml. In patients with serum creatinine concentrations of 5-17 mg/dl, serum Ep ranged from 18-115 mU/ml (mean of 40.5) before hemodialysis. The degree of anemia was also highly variable. The wide range of Ep may have been due to variability in both the degree of anemia and residual renal function. Synthesis of Ep might be increased by the anemia or decreased secondary to impaired kidney function.

Radtke, et al (1) used a fetal mouse liver cell assay to measure the serum Ep levels of 135 patients with varying degrees of chronic renal failure; they compared these with values obtained in 59 healthy controls. A first group of renal patients received no blood transfusions or iron therapy and did not need hemodialysis. They were classified in subgroups according to residual excretory renal function indicated by creatinine clearance (Cr Cl): 2-9 ml/min, 10-19 ml/min, 20-29 ml/min, 30-39 ml/min, and 40-90 ml/min. A second group of patients had endstage renal disease (ESRD) and were investigated two to six months before the onset of regular hemodialysis, immediately before, and two to six months after the first hemodialysis. Dialysis was performed three to four times for six to eight hours a week using various disposable dialyzers. The patients received no transfusions.

The development of anemia correlated with the progression of renal insufficiency, becoming manifest in

patients with a Cr Cl below 40 ml/min/1.73 m². Between 2 and 40 ml/min, the Cr Cl and hematocrit were highly significantly correlated, but with clearances between 41-90 ml/min there was no correlation.

In this study the mean Ep concentration and hematocrit of each subgroup were compared to those of healthy controls. The Ep concentrations were significantly elevated in all subgroups. The highest value, found in patients with Cr Cl between 20-29 ml/min, was not exceeded despite worsening anemia and lower Cr Cl. Between 2-40 ml/min Cr Cl, the correlation between Cr Cl and serum Ep concentration was significant, with a parallel decrease of excretory and endocrine renal function.

In patients with endstage renal failure, hematocrit decreased before dialysis and increased after dialysis was started. However, Ep concentrations increased in the predialysis period as hematocrit fell and decreased after hemodialysis began. The lowest Ep levels were found in those patients who had received dialysis for more than six months.

Koch and Radtke, using a fetal mouse liver cell bioassay, found Ep levels in one hundred anemic hemodialysis patients to be as low as those of normal patients. This observation supports the finding that renal endocrine function deteriorates as renal excretory function deteriorates (14).

Radtke also measured Ep levels and hematocrit in 42 patients with endstage renal disease immediately before treatment and after initiation of dialysis, comparing results with those in 59 controls (15). All dialysis patients received iron therapy, but no blood transfusions or androgens had been given. Mean Ep concentrations before and after dialysis were significantly higher than those of the normal controls, while hematocrit levels were significantly lower, as expected. Radtke postulated that the high levels of Ep before dialysis were a response to the severe tissue hypoxia from anemia, as well as to fluid overload with cardiorespiratory insufficiency. This paradoxical rise in erythropoietin despite worsening renal function has been reported previously to originate from nonrenal production (16). The Ep levels fell after three months of regular hemodialysis. When the hypoxic stimulus was less severe, the Ep levels were not as greatly elevated relative to the degree of anemia. In patients who had comparable anemia without renal disease, the Ep levels were much higher. In some patients, the fall in Ep after dialysis was started may have resulted from worsening intrinsic renal failure.

In endstage renal failure, the regulatory feedback between hypoxia and Ep continues, but it operates at a lower

level. The rise in hematocrit that occurs with hemodialysis does not appear to be due to an increase in renal or extrarenal Ep, because Ep levels continue to fall.

De Klerk, et al reported erythropoietin titers in patients with chronic renal failure: 45 were receiving conservative therapy, and 54 were on maintenance hemodialysis (17). Using a fetal mouse liver cell bioassay, they found that Ep levels were significantly below normal in both groups. The causes of renal failure seemed to make a difference. A significant inverse relationship between Ep level and hemoglobin concentration was found in predialysis patients with chronic glomerulonephritis but not in patients with nonglomerular disease. Ep levels did not differ significantly between anephric dialysis patients and the nephric patients dependent on blood transfusions. However, the dialysis patients dependent on transfusions had significantly lower Ep and hemoglobin levels than those patients who were not dependent on transfusions. In the latter group, Ep levels correlated positively with the level of hemoglobin, a correlation not found in those patients dependent on transfusions.

Elevated Ep concentrations found in glomerular disease suggest that in these patients the oxygen sensor mechanism is functionally intact and is located outside the glomerulus. Renal medullary interstitial cells may serve as a sensor to stimulate Ep production, by releasing renal prostaglandins into the circulation in response to hypoxia. Demonstrations by Mujovic and Fisher that indomethacin suppresses Ep production in hypoxic dogs support this theory (18). De Klerk suggested that the poor correlation between Ep and hematocrit in some patients might result from factors such as erythropoietic inhibitors, for erythropoietin is still detectable, although levels are below normal (15).

Summary

The role of erythropoietin in the anemia of renal failure can be summarized as follows:

1. Erythropoietin is probably produced in the kidney, site unknown.
2. Renal anemia develops at a creatinine clearance of less than 40 ml/min/1.73 m².
3. Erythropoietin levels correlate positively with creatinine clearance between 2-40 ml/min.
4. Erythropoietin levels are at their highest in the period immediately before dialysis.
5. Serum erythropoietin concentrations fall to levels slightly above normal controls after at least 3 months of hemodialysis.

The implications for therapy are quite important. Can erythropoietin therapy improve anemia? Van Stone administered erythropoietin to anephric, peritoneally dialyzed rats for 12 days. Treated rats had greater than three times more bone marrow red cell precursors and two times greater plasma iron turnover than did saline injected anephric rats (19). The hematocrit was also significantly higher than in uremic rats not treated with Ep, although still lower than the nonuremic control group. Studies of Ep treatment in humans are still forthcoming.

The complex role that erythropoietin plays in the development of renal failure is unfolding. When erythropoietin becomes available for clinical use, it should contribute greatly to treatment of anemia in the dialysis patient.

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