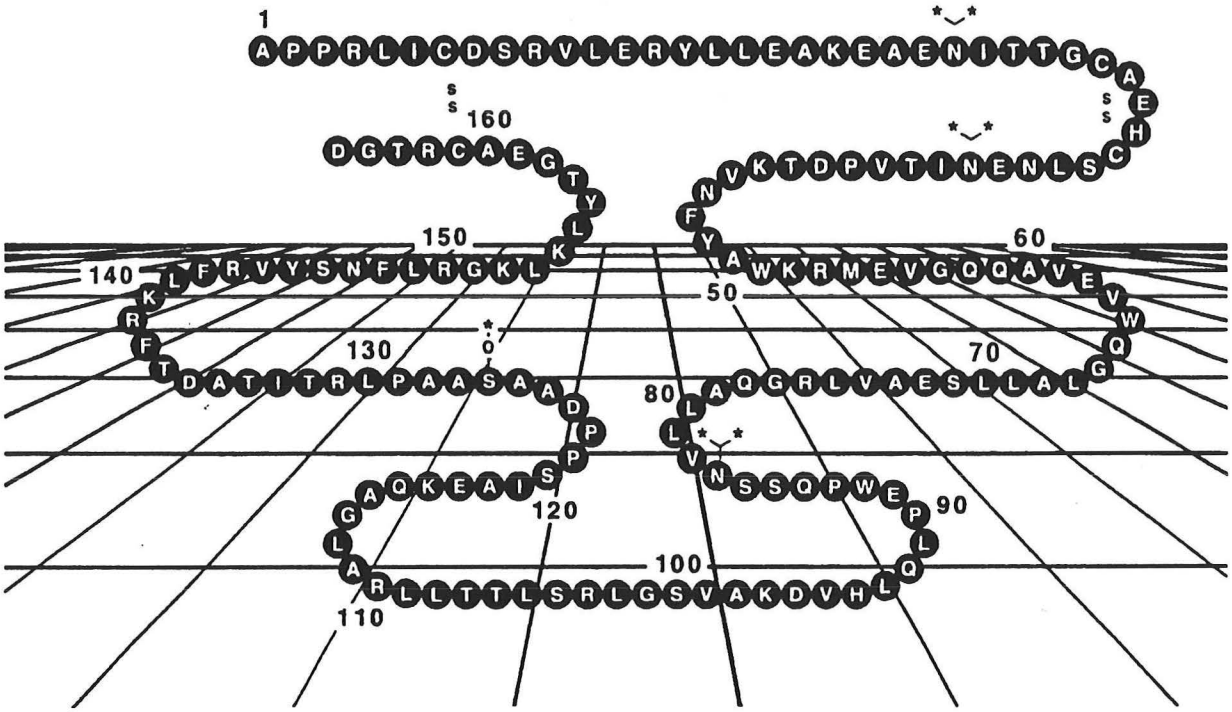
HUMAN RECOMBINANT ERYTHROPOIETIN IN THE TREATMENT OF THE ANEMIA OF CHRONIC RENAL FAILURE

MEDICAL GRAND ROUNDS



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I. Introduction

The anemia of chronic renal failure has plagued patients for millennia. It is believed to cause many of the symptoms of chronic renal failure including fatigue, dyspnea on exertion, lassitude, depression, generalized weakness, angina pectoris and impaired cerebration. Until recently, modern management of the anemia has been accomplished by administering androgens or by blood transfusions which in turn may cause their own serious complications such as chronic viral infections, acute volume overload, hyperkalemia, hypersensitivity reactions,etc. Today, erythropoietin deficiency is recognized as the major cause of the anemia of chronic renal failure. We now know that erythropoietin, or EPO, is a glycoprotein that is synthesized in the kidney, is released in response to decreased oxygen delivery the kidney (e.g. anemia or hypoxia) and regulates erythropoiesis at the level of the progenitor cells in the bone marrow. Through the technology of molecular biology the gene for erythropoietin has been isolated and cloned (64). The implications of this accomplishment are profound as evidenced by the widespread use of the genetically engineered molecule in the treatment of anemia of chronic renal failure as well as other anemias. As of this writing there is no other available product of molecular genetic technology that enjoys such widespread use in the management of so many diseases. Thus, EPO therapy has fulfilled a dream of molecular medicine: correction of a hormone deficiency is achieved by replacement of the exact molecular species through recombinant DNA technology. Indeed, administration of this agent to renal failure patients has made it is possible to completely correct the anemia of chronic renal failure and, eliminate the need for blood transfusions to maintain normal hematocrits in these patients; a truly amazing achievement. In short, we can now cure the anemia of end stage renal disease by chronic hormone replacement therapy.

The purpose of today's discussion is threefold: 1) to review the basis for anemia of chronic renal failure with special reference to erythropoietin deficiency 2) to provide an update on what is known about erythropoietin physiology and 3) to describe the current indications, clinical efficacy and side effects of recombinant human erythropoietin, rHuEPO, in patients with chronic renal failure and end stage renal disease (ESRD).

II. Anemia of Chronic Renal Failure

History

Anemia as a complication of renal failure was first described in 1836 by Richard Bright. In 1863, Jourdanet described the finding of hypoxic polycythemia in inhabitants of Mexican mountains. However, it was not until 1906 that Carnot and DeFlandre (37) postulated the existence of a hormone responsible for erythropoiesis. Subsequently, Reissman (90) demonstrated that hypoxemia in the parabiotic rat model was capable of inducing erythrocytosis although the mechanism was not elucidated in his studies. Then in 1953 Erslev (29) clearly demonstrated that infusions of anemic rabbit plasma into normal rabbits induced a significant erythrocytosis establishing for the first time the existence of a humoral erythropoietic factor. In 1957, Jacobson et al (53) showed that the kidney was the major source of this humoral factor. After many years of intensive investigation into the nature of the hormone, Miyake, Kung and Goldwasser succeeded in isolating and purifying native human erythropoietin from

pooled urine samples obtained from patients with aplastic anemia (77). This remarkable feat then led this same group to the isolation and cloning of the human erythropoietin gene in 1985 (64). Thus after nearly 150 years after it's recognition the cure for the anemia of chronic renal failure has been realized.

General Description

Anemia in chronic renal failure is a universal finding. It is normochromic normocytic anemia of moderate to severe degree generally resulting in average steady-state hematocrit values of 20-25%. Clinical studies have revealed that the anemia is hypoproliferative due to a depression of erythropoiesis and that the severity correlates roughly with the severity of the renal failure (13,65). Anemia is believed to account for many of the symptoms of chronic renal failure including fatigue, weakness, lassitude, dyspnea on exertion, angina pectoris, impaired cerebration, etc. Based on recent studies using erythropoietin to correct anemia, the list of symptoms contributed to or caused by anemia is elongating. Thus anorexia, sleep disturbances and sexual dysfunction all of which are common findings in uremic patients are reported to improve when anemia is ameliorated by hormonal replacement (23). Furthermore, objective evidence for improvement in cardiovascular endurance has been accumulated as will be discussed below (see section on Hemodynamics).

Mechanisms of Anemia

The pathophysiology of the anemia of chronic renal failure is a complex subject since a variety of factors participate in its genesis. The factors considered in this review are listed below in Table 1 and are discussed in detail below.

Table 1

ANEMIA OF CHRONIC RENAL FAILURE
Mechanisms

Primary
- **Erythropoietin Deficiency**
- **Uremic Inhibitors**

Secondary
- **Blood Loss**
- **Iron Deficiency**
- **Transfusion suppression**
- **Aluminum Toxicity**
- **Osteitis fibrosa cystica**
- **Folate deficiency**
- **Hemolysis**

Defective Erythropoiesis

Erythropoietin deficiency

The primary mechanism of anemia in renal failure is defective erythropoiesis owing to deficiency of erythropoietin (33,30a). As mentioned earlier Jacobson et al. first established that removal of the kidneys resulted

in a sharp decrease in plasma erythropoietic activity as shown below in Table 2 reproduced from their original paper.

TABLE 2

EFFECT OF NEPHRECTOMY ON ERYTHROPOIETIN PRODUCTION IN RATS

Condition of donor	Stimulus	Assay of plasma (percentage of iron-59 incorporated into RBC)
Normal	Cobalt	9·8
Nephrectomized	Cobalt	2·4
Ureters ligated	Cobalt	5·5
Control	(Saline)	2·6
Normal (hæmatocrit 25)	Bleeding	15·4
Nephrectomized (hæmatocrit 33)	Bleeding	6·0
Adrenalectomized (hæmatocrit 35)	Bleeding	11·3
Control	(Saline)	6·9

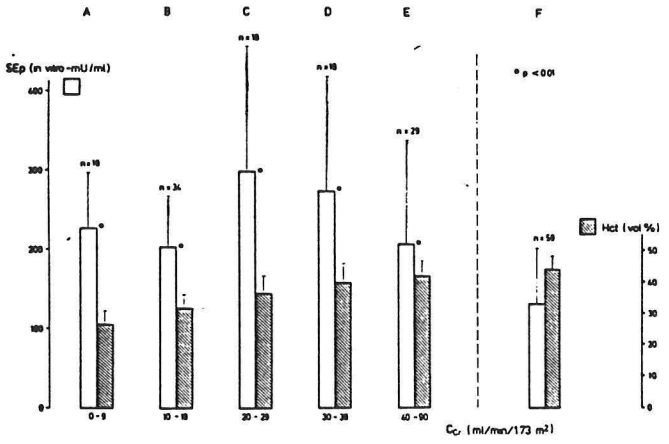
In the first group the rats were injected with 76 μM cobalt chloride immediately after nephrectomy and 12 hr. after ureter ligation. The plasma was sampled 12 hr. after cobalt administration and assayed in starved rats. In the second group the rats were bled (6 ml.) immediately after operation, the plasma sampled 7 hr. later and then assayed in starved rats. A minimum of 5 rats was included in each group.

Both cobalt and acute bleeding are known to stimulate erythropoiesis as shown in the table. Note that after either nephrectomy or 24 hours of ureteral ligation the plasma erythropoietic activity (percentage of iron-59 incorporated into RBC) is decreased.

Renal failure leads to deficiency of EPO by loss of nephron mass, thus renal endocrine deficiency parallels loss of renal excretory function (33,74,87). As shown in figure 1 below, the anemia begins to develop as the GFR falls below about 40% of normal and progressively worsens as renal failure worsens.

FIGURE 1

MEAN SERUM ERYTHROPOIETIN AND MEAN HEMATOCRIT AT VARIOUS STAGES OF RENAL INSUFFICIENCY (A-E) AND IN NORMALS (F)

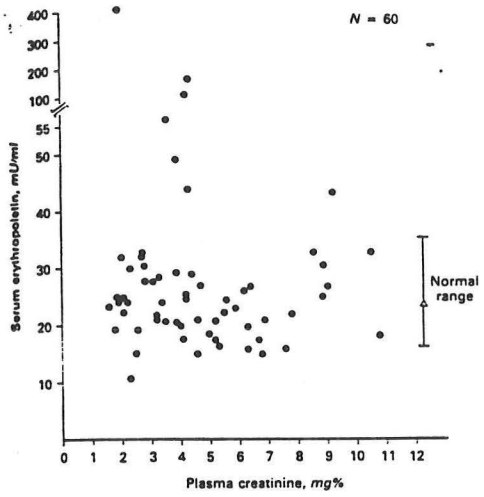


[Ref: Radtke et al. Blood 54(4):877-884, 1979.]

The circulating levels of EPO are frequently within the normal range or may even be elevated above normal despite progressive renal failure as shown in figure 2 below. However, even though erythropoietin levels may be in the "normal range" they are inappropriately low for the degree of anemia as is depicted in figure 3 taken from the work of Caro et al (15).

FIGURE 2

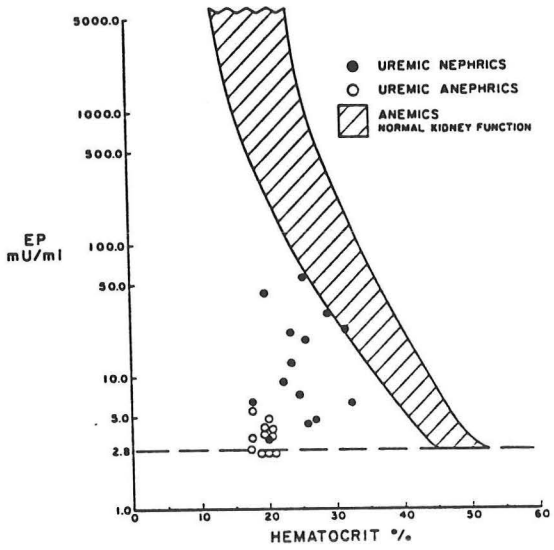
RELATIONSHIP BETWEEN SERUM EPO LEVEL AND PLASMA CREATININE IN 60 PATIENTS



[Ref: McGonigle et al. Kidney Int 25:437-44, 1984.]

FIGURE 3

RELATIONSHIP BETWEEN HEMATOCRIT AND PLASMA ERYTHROPOIETIN



[Ref: Caro et al. J Lab Clin Med 93(3):449-58, 1979.]

When ESRD is reached the hematocrit tends to stabilize in the 20-25% range. Nevertheless a low but effective output of EPO from severely damaged kidneys (and

possibly the liver) is still evident since bilateral nephrectomy in ESRD is uniformly accompanied by a further substantial fall in hematocrit from approximately 25% to 15% (104). The presence of detectable EPO at this point owes to continued, albeit low-level, hepatic output of EPO as illustrated in figure 3 (open circles). Moreover the kidney and the liver retain the ability to increase EPO production in hemodialysis patients in response to blood loss. As illustrated in table 3 below when hemodialyzed patients develop spontaneous hemorrhage, EPO levels in plasma increase in association with an acute decrease in hematocrit suggesting that the feedback loop between EPO and hematocrit is intact (115). Again it should be emphasized that the circulating level of EPO is inappropriately low for the degree of anemia observed in chronic renal failure. This is a critical point in the pathogenesis of the anemia of chronic renal failure as is borne out by the fact that the anemia can be cured in these patients when sufficient erythropoietin is administered (see below, section IV).

In summary, these data indicate that EPO is a critical factor in the generation, maintenance and magnitude of anemia of chronic renal failure.

TABLE 3

Hematologic data of eight dialysis patients experiencing minor to moderate spontaneous hemorrhages of variable duration.

	Hematocrit (%)		Corrected Reticulocyte count %		Plasma Erythropoietin mU/ml		Observed period of bleeding (days)
	From	To	From	To	From	To	
Mean	29.40	22.04	5.38	12.44	9.15	15.70	9.7
± SD	7.03	7.30	5.61	9.32	2.07	6.15	9.6
	p = 0.0005		p = 0.0065		p = 0.006		

[Ref: Walle et al. Kidney Int 31:1205-09, 1987.]

Uremic Inhibitors of Erythropoiesis

The existence and the role of important inhibitors of erythropoiesis in the pathogenesis of anemia in ESRD is controversial. Several "uremic toxins" have been reported to inhibit erythropoiesis, presumably by interfering with the action of erythropoietin on one or more of its target cells (82a). Wallner J. (25,39,75,88,89,114,116). Evidence from both experimental and clinical studies support the suggestion that erythropoietic inhibitors are present and participate in the genesis of the anemia (33,70,88). First, Radtke et al (88) have shown that improvement in anemia despite a reduction in erythropoietin levels occurs in ESRD patients after initiation of hemodialysis. As shown below in figure 4 serum erythropoietin levels decreased after 3-27 months of hemodialysis in 42 patients whereas the corresponding hematocrit levels increased.

The authors believed that this effect was due in part to removal of uremic toxins by hemodialysis. Second, a number of studies have reported that uremic serum inhibit erythropoiesis in vitro (25,39,70,72,89, ,Massry JCI 1981). For example, McGonigle et al. (74) have shown that the severity of renal failure correlates with the degree of uremic serum inhibition of erythroid colony forming units in vitro.

FIGURE 4

SERUM ERYTHROPOIETIN (S_{EP}) AND HEMATOCRIT BEFORE AND AFTER 3 TO 27 MONTHS OF REGULAR DIALYSIS TREATMENT[a]

S_{EP} mU/ml			Hematocrit % (vol/vol)		
Before	After	Normal controls	Before	After	Normal controls (N = 59)
509 ± 440	182 ± 110	136 ± 66	21.6 ± 3.6	28.7 ± 4.9	42.7 ± 3.9
← $P < 0.001$ →	← $P < 0.01$ →		← $P < 0.01$ →	← $P < 0.001$ →	
← $P < 0.001$ →			← $P < 0.001$ →		

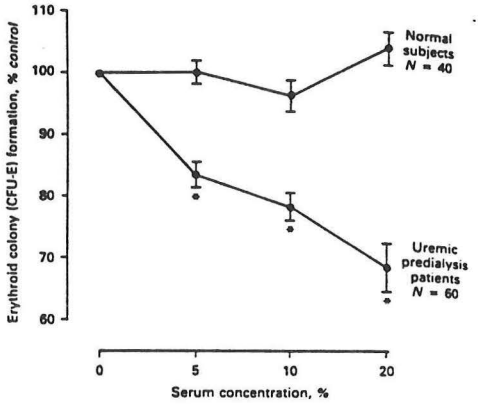
[a] Values are the means ± SD.

[Ref: Radtke et al. Kidney Int 17:382-87, 1980.]

As shown in figure 5 below, progressively increasing concentrations of uremic predialysis plasma inhibit in vitro erythroid colony formation as compared to normal plasma. In these patients, serum erythropoietin levels in uremic patients were significantly higher than normals but did not correlate with serum creatinine. In contrast, erythropoietin levels increased exponentially in anemic patients with normal renal function and colony erythroid colony forming units were markedly stimulated by normal serum. Furthermore, Wallner and Vautrin (116) have shown that uremic serum inhibitory activity increases as renal failure worsens.

FIGURE 5

EFFECT OF UREMIC PLASMA VS NORMAL PLASMA ON ERYTHROID COLONY FORMATION IN VITRO



[Ref: McGonigle et al. Kidney Int 25:437-44, 1984.]

Third, the polyamine spermine (present in high concentrations in uremic plasma in some patients) can also inhibit erthyropoiesis in vitro as measured by erythroid colony forming units (CFUS) (58). Fourth, both peritoneal and hemodialysis are associated with stability and/or improvement in anemia in patients with ESRD without erythropoietin therapy (56). The bulk of the evidence suggests that uremic inhibitors present in serum inhibit erythroid progenitor cells in vitro. In contrast to these data other investigators find either no evidence for inhibitors or find that the inhibitory effect on cell proliferation by uremia is non-specific (24,30a,33,94) For example, uremic serum inhibition of both erythroid and non-erythroid marrow cell lines and GI tract and other

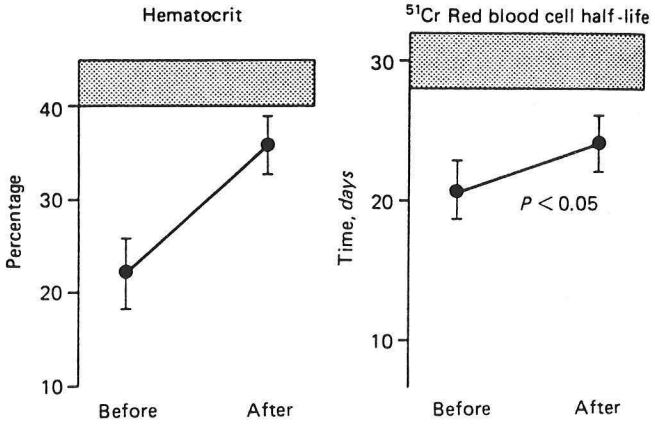
tissues have been observed suggesting that this is a non-specific effect (72). Segal et al (94) have shown that there is no difference between normals anemic ESRD and non-anemic ESRD patients with respect to the frequency of colony forming cells derived from bone marrow cultures. Moreover, pre-dialysis plasma and serum from both ESRD groups did not inhibit the growth of normal colonies in culture. However the ratio of colony forming erythroid units (CFU-E) to burst forming erythroid units (BFU-E) (an index of EPO levels in vivo stimulated erythropoiesis) is low in anemic uremic individuals because of a relative lack of erythropoietin. Furthermore in view of the fact that the effects of inhibitors (if present and active) can be overcome by administration of adequate doses of recombinant human erythropoietin, or rHuEPO, it has been suggested that the role of inhibitors in vivo is negligible. A preliminary report by Haley et al supports this contention (48). Future studies aimed at demonstrating inhibition of erythropoiesis in vivo will be necessary to resolve the conflict concerning the role of uremic inhibitors of erythropoiesis.

Shortened Red Cell Survival

Red blood cell survival is shortened in uremia (33.36). However, red blood cells of uremic patients themselves are normal as evidenced by the fact that transfusion of uremic patient cells into normal individuals returns survival to normal. In striking contrast, cells from normal individuals transfused into uremic patients have a reduced life-span (Fisher in Renal Endocrinology, ed by Durm). Based on clinical studies it has been suggested that uremic toxins particularly "middle molecules" (5,00-5,000 daltons) are responsible for reducing RBC survival (56). Unfortunately, the putative toxin responsible for rendering the RBC senescent prematurely remains elusive. It is however interesting to note that replacement o erythropoietin has been associated with an increase in RBC survival as demonstrated below in figure 6 (35).

FIGURE 6

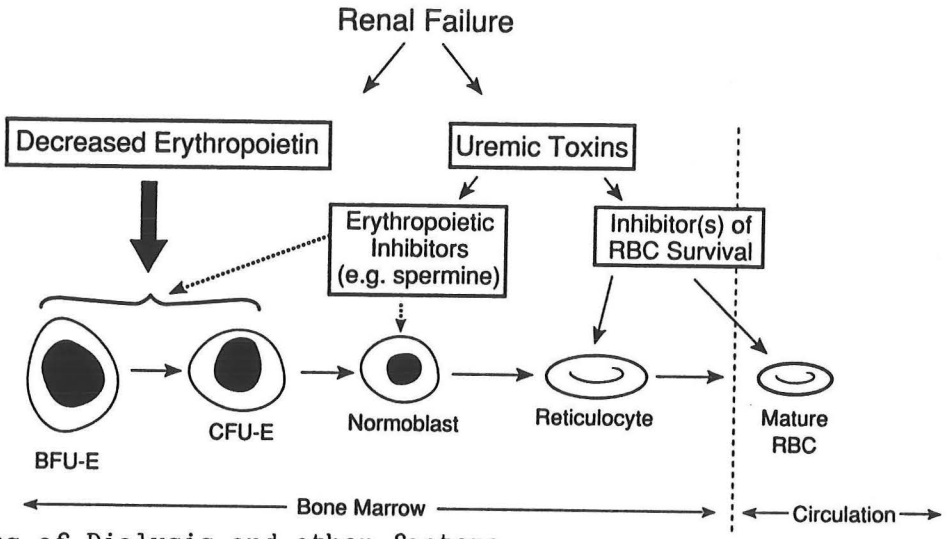
RED CELL SURVIVAL (^{51}CHROMIUM) IN 8 HEMODIALYSIS PATIENTS BEFORE AND AFTER rHuEPO THERAPY



Note that as RBC suvival increased toward normal in parallel with hematocrit. This finding raises the possibility that EPO has effects on RBC survival independent of its effects on production, however this issue will require further

studies. Figure 7 depicts an integrated model of the pathogenetic factors responsible for the anemia of chronic renal failure.

FIGURE 7
Model of Mechanisms of Anemia of Chronic Renal Failure



Effects of Dialysis and other factors

Blood Loss and Iron Deficiency

Bleeding from the access site, chronic blood loss in the dialyzer and gastrointestinal hemorrhage are relatively common occurrences in the hemodialysis population. Any one of these occurrences may lead to iron deficiency which in turn depresses erythropoiesis. The amount of blood lost during dialysis varies depending upon the type of dialyzer, the thoroughness of dialyzer rinsing, and the adequacy of heparinization. It has been calculated that up to 780 mg of iron may be lost per year in an average patient on routine hemodialysis (33) so that periodic iron repletion should be anticipated in this population. Iron deficiency may develop within 6 months to 1 year as a result of these losses, thus monitoring of iron stores by measuring serum iron, iron binding capacity and ferritin levels is performed routinely. In addition, with the advent of rHuEPO, relative iron deficiency may be a cause for persistent anemia as stored iron is removed from the reticuloendothelial system for incorporation into heme in response to rHuEPO (see below).

Aluminum toxicity

Erythroid marrow toxicity induced by chronic aluminum poisoning can result in a microcytic anemia particularly in dialysis patients who have been taking aluminum-containing phosphate binders (19). The exact mechanism of aluminum toxicity has not been elucidated but it appears that aluminum can inhibit delta aminolevulinic acid dehydratase, forroxidase and dihydropteridine reductase, it binds to transferrin and it may also be directly toxic to the bone marrow. Aluminum toxicity typically results in a microcytic, hypochromic anemia in

dialysis patients. It can be ameliorated by removing aluminum in a two step process: chelation with desferioxamine and subsequent hemodialysis to remove the chelated aluminum.

Secondary Hyperparathyroidism

Severe secondary hyperparathyroidism may contribute to anemia by suppressing erythropoiesis (75) or by osteitis fibrosa cystica-induced reduction in the erythropoietic tissue mass (104a). How PTH per se produces anemia is unclear however both a direct marrow toxic effect as well as an effect on red cell survival have been suggested (75).

Transfusion suppression

Marrow suppression by repeated transfusion of blood may exist in dialyzed patients as well since the feedback mechanism between hypoxia and renal EPO output is retained in uremia (33). Repetitive transfusions were necessary in about 25% of patients prior to the introduction of rHuEPO into our therapeutic armamentarium only one year ago. This cause of anemia should be eliminated now.

Folate Deficiency

Folate deficiency may occur in hemodialyzed patients due to folate loss across the dialyzer (33,49). This is an uncommon complication since most patients ingest sufficient amounts of folate in their diet or in the form of a supplement to prevent it (5).

Hemolysis

Both acute and chronic hemolysis may contribute to anemia in ESRD patients maintained on hemodialysis. Dialysis itself may cause hemolysis by multiple mechanisms including mechanical injury in the dialysis machine blood pump, development of anti-N-like cold agglutinins in response to formaldehyde exposure in patients dialyzed with reprocessed dialyzers (54a,Kaehny WD, Miller BE, White WL: Relationship between dialyzer reuse and the presence of anti-N-like antibodies in chronic hemodialysis patients Kidney International 12:59, 1977), and via exposure to other toxins such as chloramines (26) and copper (69). Chronic hemolysis occurs in patients on dialysis who develop medical disease known to cause hemolysis such as SLE, hypersplenism, etc.

In summary, the anemia of chronic renal failure is multifactorial. Erythropoietin deficiency the major pathogenetic factor, however erythropoietin unresponsiveness, presumably related to erythropoietic inhibitors also plays a major role in its pathogenesis. In addition, shortened red cell survival contributes. Finally, in hemodialyzed patients iron deficiency, aluminum toxicity and osteitis fibrosa cystic may also be contributing factors. Both peritoneal and hemodialysis improve anemia presumably by removing uremic toxins which inhibit erythropoietin and reduce red cell survival.

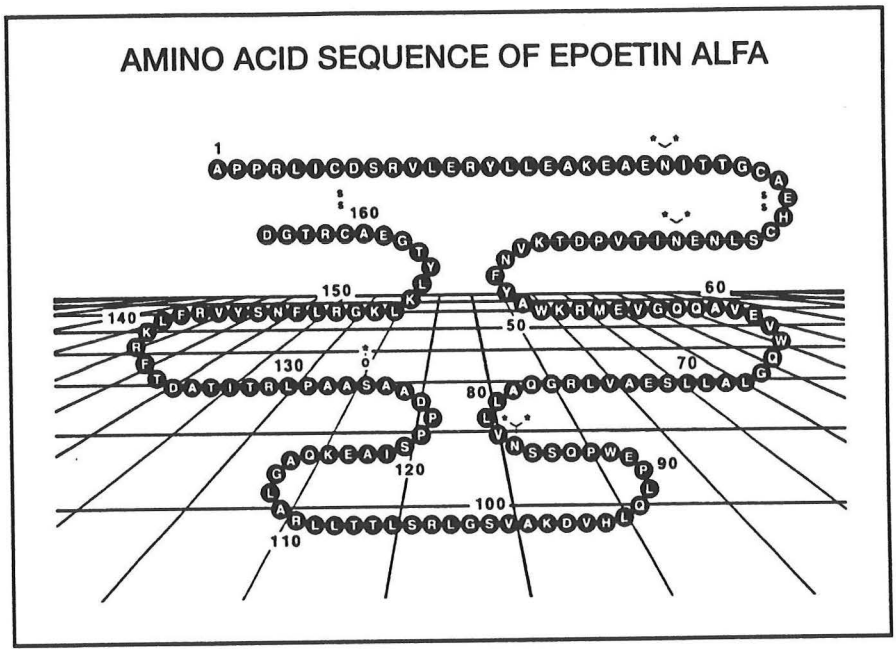
III. Erythropoietin

Physiology

Molecular Structure and sites of synthesis

Native human erythropoietin is a sialoglycoprotein composed of 166 amino acids with an approximate molecular weight of 34,000 daltons. The hormone is produced from a gene encoding 193 amino acids, the first 27 of which comprise a leader sequence which is cleaved prior to secretion from the cell. The mature molecule has four carbohydrate chains, 3 N-linked and one O-linked, about 40% of which are sialic acid containing. Although the glycosylation is not necessary for erythropoietic activity it appears to be relevant in the metabolism of the hormone. Figure 8 below is an artist's rendition of a two dimensional of the structure of the amino acid portion of the molecule showing that it contains two internal disulfide bridges. These disulfide bonds are necessary for optimal biological activity.

FIGURE 8



The approximate molecular weight of the amino acid portion of the molecule is 18,400 daltons, thus the carbohydrate component of the molecule makes up about 40% of its molecular weight. The heavy glycosylation makes it extremely difficult to crystallize this molecule hence deducing its secondary, tertiary and quaternary structures have not yet been accomplished.

Erythropoietin is synthesized in the liver and the kidney. In adults 10% of EPO is produced by the liver and 90% by the kidneys. In the kidney, the cells responsible for synthesizing the hormone reside exclusively in the cortex. and are concentrated in the juxtamedullary region. The specific cell or cells responsible for synthesis have been identified as endothelial cells of the peritubular capillaries utilizing an anemic murine kidney model (59). Utilizing murine EPO probes in anemic murine kidney sections they demonstrated co-localizion of EPO mRNA and anti-factor VIII positivity to the cytoplasm of renal cortical interstitial cells. The identification of an interstitial cell as the source of EPO synthesis has been independently confirmed by in situ hybridization

utilizing similar probes for EPO; however, in these studies endothelial cells were not clearly identified (57). The biological properties of these cell have not been elucidated since they have not yet been isolated, purified and cultured as yet. In addition it is noteworthy that EPO secreting cells proliferate in response to anemia indicating that an additional mode of regulation independent of increased EPO gene transcription is active in the case of anemia.

Molecular Genetics of EPO

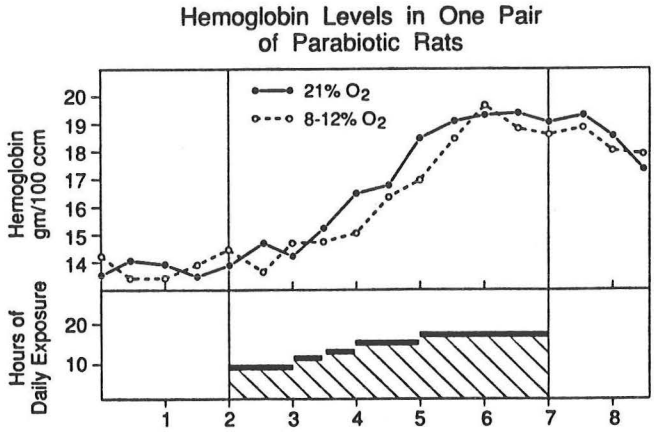
Purification of the native human hormone from urine of aplastic anemia patients in 1977 (77) triggered a burst of active research into the molecular biology of EPO culminating in the isolation, cloning and subsequent expression of the EPO gene in mammalian cell hosts (22,52,64,85). The gene for human EPO has been localized to the long arm of chromosome 7 (60). Regulated expression of the recombinant human erythropoietin gene has been accomplished and allowed for the commercial production of recombinant human erythropoietin alpha, or rHuEPO, in chinese hamster ovary cells. Furthermore, a human hepatoma cell line has proved to be an important tool for dissecting the molecular events responsible for the secretion of erythropoietin in response to hypoxic stimuli. Thus these cell lines have been shown to increase both EPO RNA and EPO protein in response to hypoxia in vitro (43).

Physiology of EPO secretion

In normal adults there is a tonic secretion of EPO from the kidney which is enhanced in response to hypoxia. Secretion of EPO appears to be stimulated by an imbalance between oxygen delivery and demand. Hypoxia defined as a decrease in oxygen-carrying capacity (anemia), hypoxemia, shunting of blood from the kidney or increased hemoglobin-oxygen affinity can stimulate EPO release. Normal circulating concentrations of EPO are about 15-20 mU/ml. In individuals with normal renal function anemia can increase this level more than five fold. However, the diseased kidney is unable to respond to anemia and rarely is the level as high as 100 mU/ml in renal failure patients. Thus there is an inverse correlation between hematocrit and erythropoietin levels in the blood of anemic patients with normal kidney function which doses not exist in patients with renal failure (see figures 2 and 3 above).

Reissman (90) first demonstrated that a humoral factor was responsible for the enhanced erythropoiesis observed in mammals. In an ingenious set of experiments employing parabiotic rats, an experimental "Siamese twin", he showed that when one rat of the pair was kept in a hypoxic chamber of 8-12% O_2 while the twin was kept in a chamber of room air both animals developed erythrocytosis despite the fact that the O_2 saturation was low only in the rat kept in the hypoxic chamber. The results of his experiments are shown below in figure 9. However it was Erslev (29) that is credited with showing that erythropoietic activity of anemic plasma was responsible for inducing this effect. As shown below in figure 10 normal rabbits injected with plasma from anemic rabbits sharply increase their reticulocyte count within 2-4 days.

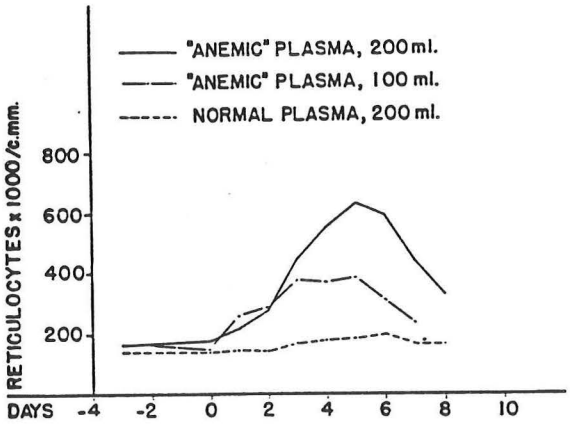
FIGURE 9



[Ref: Reissmann KR. Blood 5:372-80, 1950.]

FIGURE 10

RETICULOCYTE RESPONSE TO NORMAL AND ANEMIC PLASMA INFUSIONS IN NORMAL RABBITS



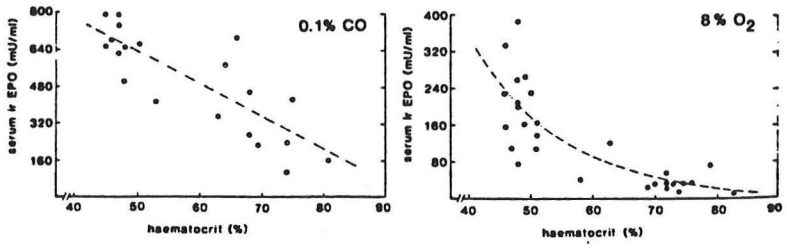
[Ref: Erslev A. Blood 8:349-57, 1953.]

This observation was subsequently confirmed and the kidney was found to be the major source of EPO in 1957 (53).

The mechanism by which hypoxia triggers erythropoietin release from the kidney remains a mystery. The relationship between hematocrit and hypoxia-induced erythropoietin response is shown below in figure 11. As illustrated in the figure, as hematocrit rises in hypoxia induced by 0.1% carbon monoxide (CO) or 8% O_2 in rats the serum EPO level increases as hematocrit decreases indicating that O_2 carrying capacity is an important determinant of EPO production.

FIGURE 11

THE RELATIONSHIP BETWEEN HEMATOCRIT AND HYPOXIA-INDUCED ERYTHROPOIETIN RESPONSE



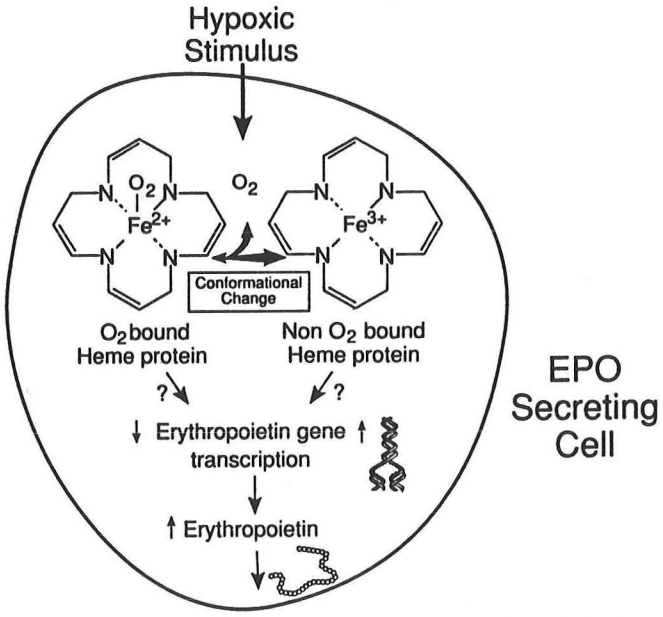
[Ref: Bauer & Kurtz. Annu Rev Physiol 51:845-56, 1989.]

Subsequent studies in experimental animals and humans have confirmed that anemic hypoxia per se is a potent stimulus to erythropoietin and are the subject of recent reviews (101,102). The location of the EPO synthesizing cells in the renal cortex makes teleologic sense based on what is known about the anatomical relationships between renal blood flow, oxygen consumption profile of various regions of the kidney and renal sodium metabolism. In 1960, Aukland and Krog showed that there was a sharp gradient between renal cortical pO_2 and renal venous pO_2 (6). Subsequent studies confirmed this finding and documented that the critical pO_2, the value below which O_2 extraction falls, is relatively higher in the renal venous effluent (35 mmHg) as compared to the renal cortex (10 mmHG). Taken together with the known anatomical relationships between efferent arteriolar and peritubular capillaries in relation to descending and ascending vasa recta, these results are consistent with O_2 shunting from peritubular capillaries to venous capillary segments in both the medulla and the cortex. In other words it is equivalent to post-glomerular shunting of blood. In hypoxic hypoxia renal oxygen consumption remains relatively constant, therefore O_2 extraction must be more efficient. Since most of the O_2 consumed by the kidney is used for sodium transport in tubular cells in the renal cortex, a reduction in O_2 delivery to the EPO secreting cells in the cortex may be explained by a reduction in shunting of O_2 from capillary blood to peritubular venule blood. If the venular endothelial cells are the site of EPO production this could perhaps explain how hypoxia increases EPO production.

Though the role of hypoxia in stimulating EPO production is undisputed, the intracellular mechanisms of O_2 sensing and signal transduction remain unknown. New information concerning this issue has been engendered by the development of molecular genetic technology. In vitro hypoxia or stimulation with cobalt chloride induce 20 to 50 fold increases in EPO mRNA in the human hepatoma cell line known as Hep 3B (43). Recent evidence suggests that the mechanism whereby hypoxia stimulates EPO synthesis is dependent upon an oxygen sensing heme protein (44). In these studies stimulation of EPO mRNA and EPO were observed in response to hypoxia or by the addition of the heavy metal cations cobalt and nickel in μM concentrations. These responses were both cycloheximide inhibitable indicating that synthesis of new protein is necessary

for enhancing EPO secretion from this experimental cell line. Co-incubation of these cells with carbon monoxide (which binds tightly to heme proteins) strongly inhibited EPO production and this inhibition could be reversed by co-incubation of the cells in 1% O_2 and cobalt. Furthermore a sharp reduction in O_2 stimulated EPO production was observed when inhibitors of heme protein synthase were added to the incubation mixture. Taken together, these data strongly support the contention that the O_2 sensing mechanism in this cell line is a heme protein. Based on this formulation a potential sensing mechanism may be envisioned as depicted in figure 12 below. In the figure O_2 is shown bound to a putative heme protein sensor which is inactive in switching on a signal which ultimately turns on the EPO gene. When this heme protein is in the deoxygenated state, such as might occur in hypoxia, the heme protein can then send a signal to switch on the EPO gene thereby enhancing its production and secretion. The intracellular signalling mechanism that transduces the signal received by this putative sensor has not yet been elucidated. However, recent studies suggest that prostaglandins, particularly prostacyclin may play an important in triggering the production of EPO (37). Possible interrelationships between prostacyclin production during hypoxemia and the putative heme protein oxygen sensor have not been investigated.

FIGURE 12
Hypothetical Mechanism of Erythropoietin Stimulation by a Hypoxic Stimulus



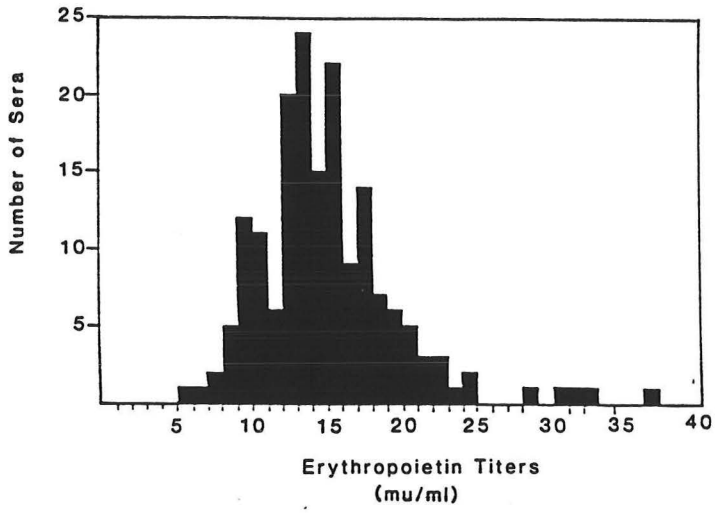
It is now known that the steady state plasma level of EPO is a function of secretion rate and metabolic breakdown of the hormone. Transient hypoxia increases EPO secretion resulting in an increase in circulating EPO levels and return of normoxia decreases the level. Thus there is a feedback loop for EPO production and secretion that regulates the level of erythropoiesis. Normal plasma levels have been determined by a specific radioimmunoassay for EPO and the results of measurements made in 175 healthy normal subjects is depicted in figure 13 below (91). As can be seen the normal level is approximately 15 mU/ml with a range of about 5-35 mU/ml. The serum level is being explored as a tool

for characterizing various erythropoietic disorders, however recent data indicate that the circulating immunoreactive EPO is a heterogeneous group which complicates the interpretation of the RIA measured levels (97).

Regulation of Erythropoiesis

Erythropoietin stimulates both differentiation and proliferation of its target cells in bone marrow. The target cell of EPO is the committed erythroid progenitor cell. EPO stimulates the differentiation of progenitor cells into globin producing cells and enhances proliferation of these cells. (For a detailed review of this process the reader is referred to the excellent discussion provided in Dr Sheehans's Grand Rounds) (95) The action of EPO accounts for the normal amount of 20ml of new red cells produced every

FIGURE 13
FREQUENCY DISTRIBUTION OF SERUM EPO TITERS IN 175 HEMATOLOGICALLY NORMAL SUBJECTS



[Ref: Rege et al. J Lab Clin Med 100:829-843, 1982.]

day by the normal adult human bone marrow. It acts by engaging a cell surface receptor which appears to activate a G_i protein and increases intracellular Calcium by as yet unknown mechanisms (18). The murine receptor has recently been cloned from a murine erythroleukemia cell line (20). It appears to have some homology to the IL-2 beta receptor gene (21). The murine EPO receptor has 507 amino acids with only a single membrane-spanning domain. Receptor affinity studies indicate that both high and low affinity receptors may be expressed in the same cell line. The density of receptors on these progenitor cells is relatively low which further complicates studies designed to elucidate its physiology. At the present time the structure of the receptor and its exact interaction with EPO are the subject of intense study, however little is known about their interaction apart from the receptor kinetic data relating to affinity and receptor number.

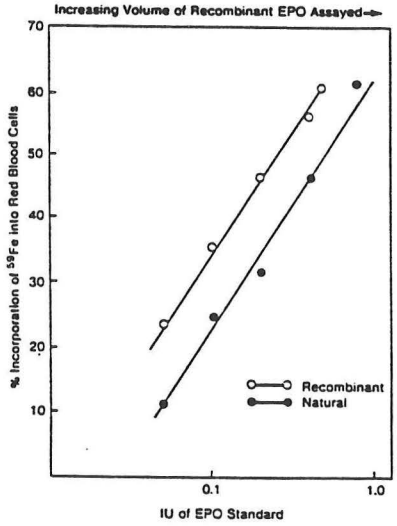
For now, precisely how engagement of EPO with its receptor communicates to the cell that its destiny is to make large amounts of hemoglobin remains a mystery.

Comparison of Native and Recombinant Human EPOs

The native and recombinant human EPOs have been carefully compared in terms of structural, immunoreactivity and erythropoietic activity levels. The results indicate that they are virtually identical (27). Both hormones stimulate erythropoiesis in vitro as evidenced by incorporation of radioiron uptake into erythroid precursor cells as shown below in figure 14. Furthermore their molecular weights and glycosylation content are identical. This similarity is due to the fact that expression of the recombinant human gene in mammalian cells allows for the appropriate glycosylation steps to take place prior to secretion of erythropoietin. Finally, the ability to stimulate erythropoiesis both in vivo and in vitro is the same for both hormone. Thus the rHuEPO is essentially identical that the native hormone both in chemical structure and in hematopoietic function. It should be mentioned that in vivo to date no antibodies to rHuEPO have been detected in any clinical study.

FIGURE 14

COMPARISON OF NATURAL AND rHuEPO IN AN IN VITRO BONE MARROW ASSAY



[Ref: Egrie et al. Immunobiol 172:213-24, 1986.]

IV. Treatment of Anemia of Chronic Renal Failure with rHuEPO

A. Patient Selection

Who should receive rHuEPO therapy? Recombinant human EPO has been administered to patients with ESRD on dialysis as well as to patients with chronic renal failure who have not yet reached end stage. Most published data on the effects of EPO come from studies performed in hemodialysis patients since it is this group that was first targeted for clinical trials with this agent. Most of the discussion that follows is taken from data obtained in the hemodialysis population, however it can be stated that in general the drug is efficacious in all patients with anemia of chronic renal failure regardless of the severity.

The major criteria for patient selection for treatment with this agent are shown below in table 4 below.

Table 4

CRITERIA FOR PATIENT SELECTION FOR rHuEPO THERAPY

Chronic renal failure
Hematocrit less than 30%
Adequate iron stores present
Absence of uncontrolled hypertension

In the first human trials patients with hematocrit levels below 25% were selected for study (31,119). Based on data from these trials the baseline iron studies were added to the criteria because of the discovery of refractoriness in patients who developed relative iron deficiency soon after initiation of EPO therapy. The presence of chronic renal failure with a hematocrit of less than 30% is generally used as the level at which to begin therapy with EPO Van Stone (111). In order to be certain that iron stores do not a limit the efficacy of EPO, serum iron, total iron binding capacity and ferritin levels are obtained in every patient. A transferrin saturation of greater than 20% and a ferritin level greater than 100 ng/ml are required before instituting EPO therapy (see below). Since hypertension has been reported in up to 25% of patients who achieve the target hematocrit of 35%, uncontrolled hypertension is a contraindication to EPO therapy.

Hematologic Response

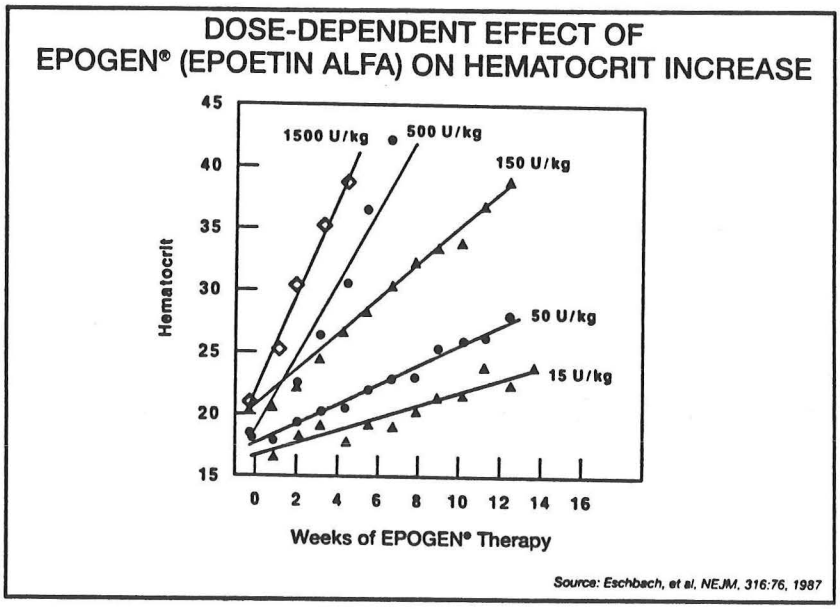
Hemodialysis

Winearls et al (119) were the first to report the effects of rHuEPO in patients with ESRD. They studied the effects of intravenous bolus infusion of 3-192 U/kg rHuEPO administered after each dialysis treatment in 10 chronically hemodialyzed anemic patients (HCT < 30%) receiving thrice weekly dialysis. They demonstrated that all patients responded to treatment with an increase in reticulocyte count by 2 weeks and an increase in hemoglobin concentration after 12 weeks of therapy. In 9 of the 10 patients the hemoglobin concentration increased from 6.1 to 10.3 g/dl. In addition, of 4 patients in this series who were transfusion dependent prior to study, transfusion requirements were eliminated by EPO therapy. Iron stores decreased along with EPO therapy, however in this study there were no incidents of iron deficiency reported. Indeed, even in those patients with normal or low iron stores (who were taking oral iron supplements) an excellent hematologic response was observed. Thus as long as iron stores were adequate or the patient ingested oral iron, the drug was 100% effective.

Eschbach et al. (31) have performed the most comprehensive studies and reported the results of combined phase I and II clinical trials carried out in the U.S in 1987. Their patients were very carefully chosen. In their study they examined the safety and efficacy of rHuEPO in 25 chronically hemodialyzed anemic

patients with the following characteristics: baseline HCT less than or equal to 25%, stable on dialysis for at least three months, normal blood pressure or controlled hypertension, absence of hemolysis or aluminum toxicity, not pregnant, not on androgen or immunosuppressive therapy, not receiving insulin therapy for diabetes and with normal liver function (AST no more than 2 times normal). They studied the dose-response relationship between EPO and hematocrit using doses ranging from 1.5-500 U/kg body weight administered as an intravenous bolus at the end of each dialysis treatment given 3 times per week. Four key points emerged from this study. First, they found that at doses of 50 U/kg or higher a consistent dose-dependent increase in corrected reticulocyte count, erythron transferrin uptake (index of in vivo erythropoiesis), and rate of rise in hematocrit occurred. The dose-response curve for this study is shown below in figure 15. As can be seen the rate of response was clearly dose dependent at 12 weeks. It is noteworthy that the hematocrit increased in all four doses and that the hematocrit doubled within four to five weeks in the highest dose used. However, despite the positive response shown for the 15 U/kg dose this was not a consistent response to therapy in this group of patients. Second, rHuEPO therapy eliminated the need for transfusions in previously transfusion-dependent patients confirming the findings of Winearls et al. Third, they discovered the association between rHuEPO therapy

FIGURE 15



and the development of relative iron deficiency. As shown in figure 16 below, when the drug is administered to patients with normal iron stores who are not receiving concomitant iron therapy, after a period of initial response to EPO, refractoriness to therapy may develop as a result of acquired relative iron deficiency.

FIGURE 16

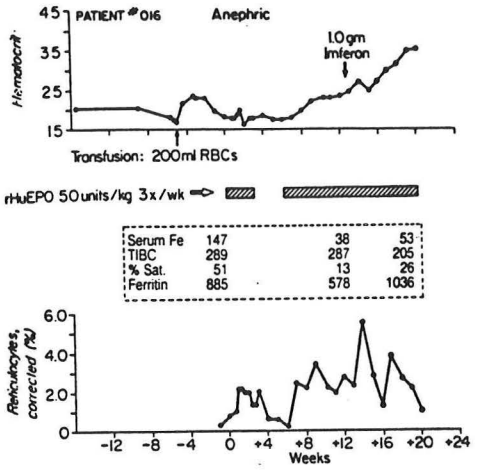


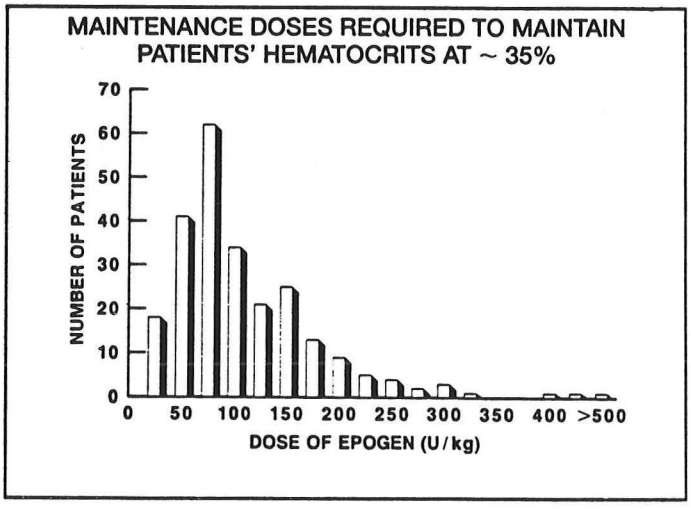
Figure 5. Relative Iron Deficiency Induced in a Patient by 50 Units of Recombinant Human Erythropoietin (rHuEPO) per Kilogram Given Three Times Weekly.

After an initial rise in the reticulocyte count and hematocrit with therapy, there was a plateau of response at weeks 10 through 12. The transferrin saturation fell to 13 percent, and consequently 1 g of iron dextran was given intravenously. After this there was a prompt reticulocytosis, and the increase in the hematocrit was reestablished.

[Ref: Eschbach et al. NEJM 316(2):73-78, 1987]

Note that after reinstitution of rHuEPO therapy at +6 weeks, when iron stores were decreased relative to baseline, the hematocrit reached a plateau but promptly increased after the administration of Imferon. This finding has led to the recommendation that iron stores be determined at the onset and monitored during course of therapy with EPO (114). Fourth, they demonstrated that discontinuation of the drug results in recurrence of anemia within 2 weeks indicating that chronic continuous therapy is needed to maintain the hematocrit. Finally they showed that virtually every patient has a positive response to the drug so long a as iron stores are adequate. Thus they confirmed the findings of Winearls and further demonstrate the dose response to the drug. These data have been expanded in a multi-center phase III clinical trial involving 300 patients in whom baseline hematocrit increased by 6% in over 97% of patients by 12 weeks of therapy.(34). In this study, the median dose required to maintain Hct in the 32-38% range was 75 U/kg thrice weekly as shown below in figure 17.

FIGURE 17



Nevertheless, it is clear that a small proportion of patients will require doses of 300 or more U/kg to maintain the target hematocrit. Indeed in this study, 17% of patients required a dose of greater than 150 U/kg. Finally it is noteworthy that in this large study the steady-state mean serum transferrin saturation and ferritin levels during therapy were significantly reduced compared to the pre rHuEPO period.

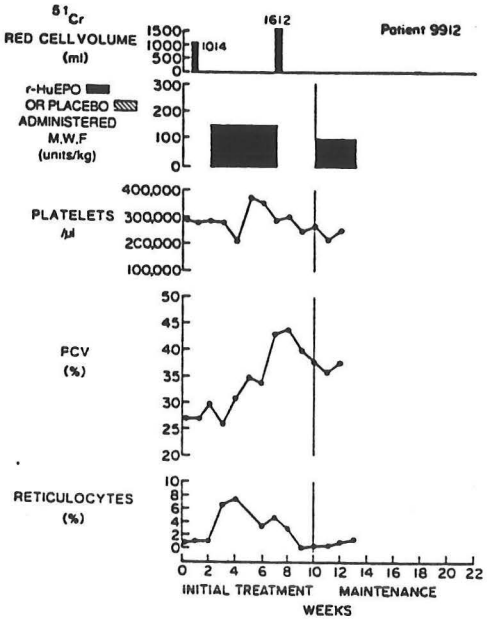
Several other short-term studies have furnished corroborative data concerning the effects of rHuEPO (14,84). In all studies the drug has been administered thrice weekly at the end of each dialysis treatment usually as an intravenous bolus. It should be noted however that the drug is just as efficacious when administered subcutaneously. Thus, there is no doubt that rHuEPO is an effective means of correcting the anemia of ESRD and that it has the added benefit of eliminating the need for transfusion therapy for a large number of patients.

Predialysis

Data on the efficacy of rHuEPO in the predialysis patients is similar to that furnished from the dialysis patient studies. Thus, Stone et al. (105) have demonstrated a positive response in predialysis patients with creatinine clearances in the range of 10-30 ml/min receiving rHuEPO in doses of 150 U/kg (given thrice weekly as an intravenous bolus) by 8-10 weeks of therapy. The response to rHuEPO in a representative patient from their study is shown below in figure 18.

FIGURE 18

HEMATOLOGIC EFFECT OF rHuEPO IN A PRE-DIALYSIS PATIENT



[Ref: Stone et al. Am J Med Sci 296(3):171-9, 1988.]

As can be seen red cell mass, hematocrit and reticulocyte count all increased dramatically in response to rHuEPO therapy and declined with interruption of

treatment: findings similar to those seen in dialysis patients. These findings have been confirmed by Lim et al. (61). In both of these studies the drug was given intravenously, however responses to subcutaneous administration are similar and this will undoubtedly become the route of administration of rHuEPO for predialysis patients in the future. EPO reaches peak levels in 8-12 hours after subcutaneous injection and levels are maintained fairly constant for 12-16 hours (38).

Dosage of rHuEPO

Based on these and other studies involving similar patient groups the recommended doses of rHuEPO for initiation and maintenance therapy in dialysis patients have been published. Since the half life in blood ranges between 5-9 hours in humans on hemodialysis and up to 11 hours in patients on CAPD, the drug can be given thrice weekly after dialysis as has been demonstrated in the preceding studies (38). The starting dose is 50-100 U/kg body weight three times per week administered at the end of dialysis and continuing until hematocrit increases to a target range of 33% with a maximum Hct of 36%. If the response is inadequate after 2-6 weeks the dose is doubled until a desired response is reached or until a dose of 300 U/kg body weight is reached. If no response occurs at this point investigation into causes of resistance to therapy must be considered. After achieving the target hematocrit the dose is reduced by 12-25 U/kg and titrated to maintain the Hct within the target range. Current guidelines for rHuEPO therapy including monitoring information are summarized below in Table 5.

TABLE 5

Guidelines for Recombinant Human Erythropoietin Administration

Patient selection criteria
- Hematocrit: ≤0.30 or transfusion dependent
- Iron indices: serum ferritin >100 ng/mL and/or transferrin saturation >20%

Therapeutic goal
- Hematocrit: 0.32 to 0.38

Dose/response

Dosage	Hematocrit Increases
150 U/kg 3×/wk	0.018/wk
50 U/kg 3×/wk	0.010/wk

Reduce dose when hematocrit reaches 0.30, then adjust maintenance dose by 12.5 to 25 U/kg as needed

Monitoring guidelines

Test	Initial Frequency	Duration	Thereafter
Hematocrit	Weekly	12 wk	Bimonthly
Reticulocytes	Weekly	1 to 4 wk	As needed
Iron, TIBC, serum ferritin	Monthly	3 mo	Bimonthly
BP	3×/wk	Throughout	
Potassium	2×/mo	Until stable	Monthly

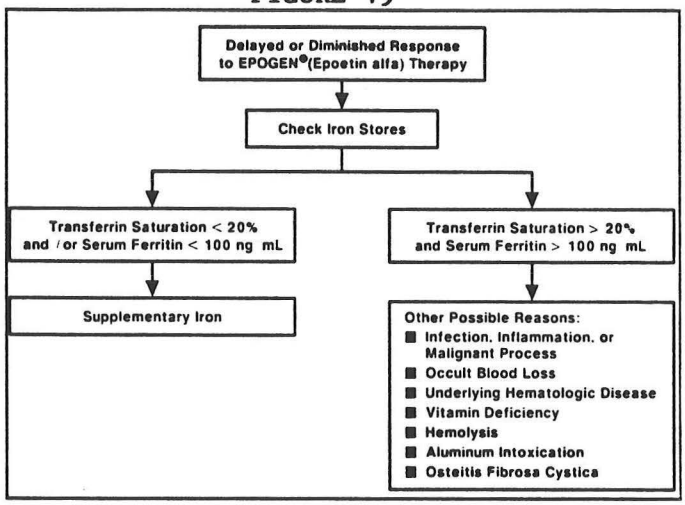
Iron supplementation
- Goal: percent transferrin saturation >20%
- Therapy: oral iron 3×/d or IV iron dextran, 0.5 g

Abbreviation: TIBC, total iron-binding capacity.

Resistance to therapy

Several of the above cited studies have found that certain patients remain relatively resistant to even large doses of rHuEPO (61-63,84,105). The reasons for lack of response may include iron deficiency (as cited above), acute inflammation and surgery (61,104a), aluminum toxicity and osteitis fibrosa cystica (46,50,109), occult blood loss, hemolysis and underlying hematologic disease (34), uremic erythropoietic inhibitors (104a) and as yet unknown factors (3). Interestingly, some hemodialysis patients refractory to rHuEPO respond when switched to chronic ambulatory peritoneal dialysis which has led to the suggestion that a dialyzable middle molecule acts as an erythropoietic inhibitor in some patients (104a). The approach to resistance is schematically illustrated below in figure 19. Iron supplementation is a key management parameter that must be prospectively assessed in patients receiving EPO since this is a common cause for apparent resistance to therapy (114).

FIGURE 19



Hemodynamics

Chronic stable anemia in patients with end stage renal disease is associated with increased cardiac index and decreased total peripheral vascular resistance (18a,79) and transfusion results in an increase in blood pressure in these patients accompanied by and increase in total peripheral vascular resistance (TPR). In both experimental animal models and in ESRD patients, correction of anemia with rHuEPO is often accompanied by an increase in blood pressure, TPR and a decrease in cardiac index (CI) (24a,54). Data on rHuEPO in humans concerning the issue of hemodynamic response have been reviewed by Nonnast-Daniel et al (82). Table 6 below is taken from their work.
As can be seen, utilizing a variety of both invasive and non-invasive techniques a fairly consistent pattern emerges: CI decreases in about half the studies whereas TPR and BP increase in most. Why CI does not decrease in some patients remains unknown but is in contrast to the response in non-uremic anemic patients undergoing correction of anemia. Based on these data, three possible mechanisms have been proposed to explain the increase in BP after correction of anemia in ESRD patients.

TABLE 6

Hemodynamic changes induced by rhEPO

Author	Method	Patients, n	CI	TPR	BP
Deschodt et al., 1988 [6]	dye dil.	4	↓	↑	↑
Buckner et al., 1989 [4]	echo	7	(↓)+	↑	↑
Hori et al., 1988 [14]	echo	6	↓	↑	↑
Abraham et al., 1989 [1]	echo	8	±	±	±
Akiba et al., 1989 [2]	Swan-G.	6	↓	↑	±
		6	±	↑	↑
Nonnast-Daniel et al., 1989 [21]	Swan-G.	5	↓	↑	(↑)
		2	±	↑	↑↑

CI = Cardiac index; TPR = total peripheral resistance; BP = blood pressure.

First an increase in blood viscosity owing to the increase in packed cell volume could increase resistance to flow. Second, a reduction in hypoxia-induced vasodilatation by restoration of O_2 carrying capacity could result in vasoconstriction and thereby an increase in TPR. Third a failure in reduction of cardiac output could play a role in some patients particularly if TPR remains unchanged or increases. In addition two preliminary reports suggest that the adrenergic nervous system may be activated by increasing hematocrit by rHuEPO therapy (8,41). Finally it has been suggested by Moncada (70a) that nitric oxide EDRF which binds tightly to hemoglobin may be relatively deficient when hemoglobin levels increase thereby reducing the vasodilator activity of this substance further potentiating peripheral resistance.

The effect of correcting anemia on exercise capacity has also been investigated. Two published studies have shown an increase in maximal oxygen uptake after rHuEPO treatment (92,106) after correction of anemia. In a recent study performed in our institution by Dr. Jeffrey R. Thompson of the Nephrology Unit and Dr. James Stray-Gunderson of the Department of Orthopedics similar results have been obtained in chronically hemodialyzed patients who have been treated with E rHuEPO for 12 weeks in whom the hematocrit increased to the 35-49% range. In addition to the increase in maximal O_2 uptake, cardiac index was found to increase in during exercise to a greater extent after rHuEPO as compared to the increase observed before rHuEPO therapy. In addition studies in predialysis patients reveal that increases in TPR and BP are similar to hemodialysis patients, however cardiac index may not decrease (51,93). The increases in maximal O_2 uptake and cardiac index induced by exercise may explain in part the fact that patients successfully treated with EPO generally report an improvement in their quality of life.

Quality of Life

The symptoms of anemia of chronic renal failure may contribute to the relatively poor objective quality of life experienced by this patient population. For example the weakness, dyspnea, fatigue and anorexia may impact significantly on the ability to work and their senses of physical and psychological well-being. Low exercise capacity in ESRD patients has been well documented. The reasons for low exercise capacity are multiple and complex. Besides anemia

deconditioning, cardiac disease, hypertension and possibly uremia-associated abnormalities in mitochondrial enzyme activities may play a role.

Several studies have reported improvement in exercise capacity after amelioration of anemia by rHuEPO in hemodialysis patients (51,68,92,93,106). Based on the concomitant increases in hematocrit and maximal O_2 uptake it has been concluded that in the short-term, rHuEPO results in an objective improvement in exercise capacity. However, even though demonstrable increases in exercise capacity occur the improved levels are still near the low end of the normal or frankly subnormal and far below that of a trained athlete (3 to 5 fold lower). In addition to these effects, Grutzmacher et al (46) have shown improvement in myocardial function in two patients on long-term hemodialysis and improvement in symptoms in 16 patients all of whom had symptomatic heart disease.

Subjective improvement in symptoms of anemia has been surveyed in many studies. A representative sample of the type of data obtained is illustrated below in table 6 from Eschbach et al (34).

TABLE 7

EFFECT OF RECOMBINANT HUMAN ERYTHROPOIETIN ON PATIENTS' FUNCTIONAL IMPAIRMENT, ENERGY AND ACTIVITY LEVEL (n=130)

	Baseline	Second Evaluation*	Third Evaluation†
Hematocrit, mean	0.237	0.342	0.339
Functional impairment			
Normal, no complaints; able to carry on normal activity (Karnofsky), *% of patients*	25.9	44.5‡	43.5‡
Activity level			
Very or mostly active, *% of patients*	19.8	37.3‡	35.5‡
Energy level			
Patient reporting very full of energy or fairly energetic most of the time, %	25.9	45.4‡	48.1‡
Patients reporting low energy or no energy at all, %	46.2	23.2‡	22.2‡
Nottingham Health Profile score§	47.0	31.5‡	27.7‡

* Approximately 6 months after initiation of rHuEpo therapy.
† Approximately 10 months after initiation of rHuEpo therapy.
‡ $P \leq 0.01$ compared with baseline.
§ Scores, 100 = complete limitation; 0 = no physical limitation.

[Ref: Eschbach et al. Ann Int Med 111:992-1000, 1989]

In addition to the effects on sense of well-being , activity level, energy level and health profile scoring other effects have been reported. For instance improvement in cognitive function (80,81), visual-motor tracking and audio-verbal learning (115a). These data support the view that the correction of anemia will have long-term implications on the quality of life of ESRD patients in the future. Most of these data have been reported in small numbers of patients and some appear only in preliminary form at this time. Moreover, despite these initial positive results it remains to be determined whether the correction of anemia by rHuEPO results in sustained improvement in these parameters and most importantly whether improved rehabilitation and return to

productive lifestyle will eventuate.

Effects on Renal Function

There is a paucity of information concerning the effects of anemia on renal function. Interest in the role of anemia and renal failure stems from the fact that adaptations in renal hemodynamics occur in response to both the development of renal failure and to anemia. Whereas it might be imagined that anemia would exacerbate renal failure, experimental animal studies suggest that anemia protects the kidney from progressive renal failure since correction of anemia in uremic rats (induced by renal ablation) accelerates the progression of renal failure (42). In contrast, renal function studies in humans with chronic renal failure (predialysis) indicate that correction of anemia does not accelerate progression of renal failure (40,63). Based on these preliminary studies it is not known whether correction of anemia might even preserve renal function. In this regard the studies by Lim (62) were suggestive but not conclusive that progression of renal failure, as estimated by the reciprocal of the serum creatinine concentration, might actually be slowed by correction of anemia. This has implications for delaying onset of dialysis patients who will ultimately develop ESRD.

Effects on dialysis

Clearance of urea and other small molecular weight solutes from plasma across a dialysis membrane is determined by a complex interaction of various physical factors including blood flow rate, membrane composition and flow geometry in blood and dialysate compartments of the artificial kidney. It has been postulated that increasing hematocrit with the attendant relative reduction in plasma water might alter the efficiency of clearance of solutes such as urea necessitating increases in the type, amount and/or duration of dialysis (99,100). However, clinical experience with rHuEPO has not supported this theoretical possibility since only minimal alterations in steady-state BUN and serum creatinine concentrations have occurred during rHuEPO therapy. (1,34,78,82a,86). Although slight decreases in potassium and phosphorus clearance have been reported the and hyperkalemia does develop with rHuEPO (34) these elevations are believed to be do to increased dietary intake owing to improved appetite and well-being. Importantly, dialysis prescriptions do not need to be altered to accommodate the increase in hematocrit in order to maintain adequacy of dialysis.

Adverse Effects of rHuEPO

The beneficial effects of rHuEPO are undisputed; furthermore, it is now widely accepted as the therapeutic agent of choice for managing anemia in renal failure. However as has been reported in virtually all studies there are significant adverse effects that deserve special consideration .

The most common adverse side effect of rHuEPO is hypertension. This complication occurs in roughly 25% of patients and is usually moderate although hypertensive crisis has been reported (110). (The possible mechanisms of hypertension have been discussed, see above.) It is especially likely to develop in patients treated with antihypertensive agents while on hemodialysis prior to beginning therapy with rHuEPO (117). Nevertheless the development of hypertension does not preclude therapy with rHuEPO but patients with hypertension

should be monitored closely and the dose should be adjusted gradually and BP treated accordingly as the hematocrit rises. The rise in blood pressure in patients with hypotension may in fact be beneficial as has been pointed out by Watson (117).

Grand mal seizures have been reported in a minority of patients treated with rHuEPO usually in association with the development of hypertension. Whether rHuEPO causes seizures by a mechanism other than an increase in blood pressure remains to be determined as controversy still exists on this issue. In view of the fact that HCT may rise abruptly during dialysis owing to volume removal, it is possible that hypertension is precipitated by an increase in peripheral resistance during this period precipitating hypertensive encephalopathy and seizures (82,107);

Vascular access thrombosis has also been observed in patients as hematocrit increases with rHuEPO treatment. The incidence of this complication was 11% in a large multi-center trial (34). In most cases graft occlusion occurred in patients with prosthetic grafts. These grafts are much more prone to thrombosis than Cimino-Brescia arterio-venous fistulae and when considered in relation to the rate of thrombosis of these grafts in non-rHuEPO treated anemic patients, the authors of the multi-center trial concluded that the incidence of graft occlusion was not increased by rHuEPO. Nevertheless it remains an important concern since alterations in blood rheology engender thrombus formation in the vascular access of patients with non-laminar graft blood flow. Both prosthetic and primary A-V shunts may exhibit abnormalities in blood flow. Importantly, there has not been an increase in the incidence of myocardial infarction or stroke in rHuEPO treated patients studied so far. However, as more patients are treated with rHuEPO a better estimation of the magnitude of this problem will undoubtedly emerge.

Finally, other adverse side effects reported with rHuEPO include hyperkalemia in hemodialysis patients presumably from increased dietary intake (117), alterations in protein S and C levels (118) and early thrombosis of renal allograft artery (121).

Economics

There are approximately 125,400 dialysis patients in the United State, 25% of whom may be transfusion-dependent and the vast majority of whom have HCT levels below 30% making them candidates for EPO. In addition there are an estimated 234,000 patients with chronic renal failure about 35% of whom may be candidates for rHuEPO therapy. The FDA has approved the drug for use in both of these populations. Furthermore, clinical trials of EPO therapy for anemia in a variety of other circumstances including Rheumatoid arthritis, cancer, sickle cell anemia, and AIDS are underway (45,67,76).

The current wholesale cost of EPO is $10 per 1,000 U and the Health Care Finance Administration (HCFA), which establishes policy for reimbursement for ESRD, allows $40 for doses up to 10,000 units and $70 for doses above 10,000. (There is no policy established for payment of predialysis patients.) At a dose of 50 U/kg three times per week for a 70 kg person this calculates to $5,460 per year and for all dialysis patients would amount to $668,776,000 annually. This is a gross estimate since some patients will require much less and others much more for maintenance therapy. It is also worth noting that the reimbursement by HCFA will not be sufficient to cover the cost of some patients whereas it will more than afford the cost for others. The key question is whether or not the

costs of the drug will result in sufficient benefits in terms of: lessening costs of blood transfusions and their attendant morbidity, reducing the incidence of complications of anemia (e.g. angina peripheral ischemia, etc.), improving cardiovascular performance, exacting a higher rate of rehabilitation of ESRD patients, and increasing the time to onset of dialysis in the predialysis patients. A large number of variables must therefore be taken into account to perform a valid cost-benefit analysis. Sheingold et al (96) have estimated the cost-benefit of long-term rHuEPO therapy in the dialysis and predialysis populations in the U.S and Canada and have concluded that rHuEPO is cost-effective in the long-run. It will result in substantial savings in terms of offsetting costs of dialysis when transfusions, hospitalizations, improved transplant success and costs of androgen therapy are taken into consideration. In the predialysis stage the benefit will also be justified presumably by delaying dialysis.

The recommendations for rHuEPO initiation and maintenance are changing based on further clinical experience and on the cost considerations (7). Lower doses than prescribed by the clinical studies may be used to initiate and maintain therapy than originally proposed. One year after release of rHuEPO therapy the average dose range for maintenance therapy is 2800-3200 units per treatment. In view of this evolution in practice it seems likely that many of the patient outcomes and therapeutic guidelines detailed in this Grand Rounds are likely to change with time. Much more information is needed before the application of this agent to renal failure patients is streamlined and effective.

Summary and Conclusions

In summary, rHuEPO is an effective new therapeutic agent forged from the tools of modern molecular medicine. It has the capability of curing the anemia of chronic renal failure and is effective in nearly 100% of end stage renal disease patients. The currently known adverse side effects of the drug are now well established and can be effectively prevented or managed by judicious use of the drug and by paying attention to blood pressure response and body iron stores. Nearly all ESRD patients are being treated with this drug and it is likely that many predialysis patients with chronic renal failure will be treated with it shortly. The cost of the drug in dollars is considerable but it is expected to be partially defrayed by savings accrued from better patient well-being, reduction in the incidence of complications of anemia, treatment of anemia with blood transfusions and finally by competition in the medical marketplace. Finally, it rings true that molecular biology is not only big business in the laboratory but also in the arena of clinical medicine as exemplified by the development and application of this novel therapeutic agent.

REFERENCES

1. Acchiardo SR, Quinn BP, Burk LB, Moore LW. Are high flux dialysis and erythropoietin treatment in a collision course? ASAIO Transactions 35:308-310, 1989.

2. Adamson JW, Eschbach J, Finch CA. The kidney and erythropoiesis. Am. J. Med. 44:725-733, 1968.

3. Adamson JW, Egrie JC, Haley NR, et al. Who do some hemodialysis patients (HDP) need large doses of recombinant erythropoietin (rHuEPO)? Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program p. 47A, 1989.

4. Akiba T, Kurihara S, Katoh H, et al. Hemodynamic changes of hemodialyzed patients by erythropoietin (EPO) treatment. Am. Soc. Nephrology 21st Annual meeting, Abstracts on Recombinant Erythropoietin p. 7, 1988.

5. Andersen, KEH. Folic acid status of patients with chronic renal failure maintained by dialysis. Clin. Nephrology 8:510-513, 1977.

6. Aukland K, Krog J. Renal oxygen tension. Nature 4751:671, 1960.

7. Avram MM, Bennett WM, Blagg CR, et al. An EPO progress report. Dialysis & Transplantation 19:280-283, 1990.

8. Baldamus CA, Pollok M, Steffen HM, et al. Adrenergic system in renal anemia, corrected with recombinant human erythropoietin (rhEPO). Am. Sco. Nephrology 22nd Annual Meeting, Abstract Program p. 208A, 1989.

9. Bauer C, Kurtz A. Oxygen sensing in the kidney and its relation to erythropoietin production. In: Annual review of physiology, JF Hoffman ed., Annual Review Inc. Palo Alto, volume 51, pp. 845-856, 1989.

10. Bedogna V, Valvo E, Alberti D, Maschio G. Systemic and renal effects of benazepril (BNZ), a new ACEI, in patients with chronic glomerulonephritis. Am. Soc. Nephrology, Abstract Program p. 47A, 1989.

11. Brown AL, Tucker B, Baker LRI, Raine AEG. Seizures related to blood transfusion and erythropoietin treatment in patients undergoing dialysis. Br. Med. J. 299:1258-1259, 1989.

12. Buckner FS, Eschbach JW, Haley NR, et al. Correction of the anemia in hemodialysis (HD) patients (pts) with recombinant human erythropoietin (rHuEPO): Hemodynamic changes and risks for hypertension. Am. Soc. Nephrology 21st Annual meeting, Abstracts on Recombinant Erythropoietin p. 2, 1988.

13. Callen IR, Limarzi LR. Blood and bone marrow studies in renal disease. Am. J. Clin. Path. 20:3-23, 1950.

14. Canaud B, Polito-Bouloux C, Garred LJ, et al. Recombinant human erythropoietin: 18 months' experience in hemodialysis patients. Am. J. Kidney Dis. 15:169-175, 1990.

15. Caro J, Brown S, Miller O, et al. Erythropoietin levels in uremic nephric and anephric patients. J. Lab. Clin. Med. 93:449-458, 1979.

16. Carozzi S, Nasini M, Schelotto C, et al. Bone marrow erythroid precursor cytoplasmic (BMEP) Ca++ regulates the response to human recombinant erythropoietin (rHuEPO) in hemodialysis (HD) patients. Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program, p. 102A, 1989.

17. Chaplin H, Mollison PL. Red cell life-span in nephritis and in hepatic cirrhosis. Clin. Science 12:351-360, 1953.

18. Cheung JY, Robishaw JD, Whitfield CF, Miller BA. Pertussis toxin (PT) sensitive GTP-binding proteins (G-proteins) are involved in signal transduction of erythropoietin (EPO). Am. Soc. Nephrology, Abstract Program, p. 25A, 1989.

18a. Coleman B. Hemodynamics of uremic anemia. Circulation 45:510-511, 1972.

19. Cronin RE. Aluminum toxicity syndromes in chronic hemodialysis. Medical Grand Rounds Dec. 10, 1987.

20. D'Andrea AD, Lodish HF, Wong GG. Expression cloning of the murine erythropoietin receptor. Cell 57:277-285, 1989.

21. D'Andrea AD, Fasman GD, Lodish HF. Erythropoietin receptor and interleukin-2 receptor ß chain: A new receptor family. Cell 58:1023-1024, 1989.

22. Davis JM, Arakawa T. Characterization of recombinant human erythropoietin produced in Chinese hamster ovary cells. Biochemistry 26:2633-2638, 1987.

23. Delano BG. Improvements in quality of life following treatment with r-HuEPO in anemic hemodialysis patients. Am. J. Kidney Dis. 14:14-18, 1989.

24. Delwiche F, Garrity MJ, Powell, JS, et al. High levels of the circulating form of parathyroid hormone do not inhibit in vitro erythropoiesis. J. Lab. Clin. Med. 102:613-620, 1983.

24a. Deschodt B, Granolleras C, Alsabadini B, et al. Changes in cardiac output, blood pressure and peripheral resistance

following treatment of renal anemia by recombinant human erythropoietin. Nephro. Dial. Transplant 3:494, 1988.

25. Dunn CDR, Trent D. The effect of parathyroid hormone on erythropoiesis in serum-free cultures of fetal mouse liver cells (41108). Proc. Soc. Exp. Biol. Med. 166:556-561, 1981.

26. Eaton JW. Chlorinated urban water: A cause of dialysis-induced hemolytic anemia. Science 181:463-464, 1973.

27. Egrie JC, Strickland TW, Lane J, et al. Characterization and biological effects of recombinant human erythropoietin. Immunobiol. 172:213-224, 1986.

28. Eichner ER, Paine CJ, Dickson VL, Hargrove MD. Clinical and laboratory observations on serum folate-binding protein. Blood 46:599-609, 1975.

29. Erslev A. Humoral regulation of red cell production. Blood 8:349-357, 1953.

30. Eschbach JW, Adamson JW, Cook, JD. Disorders of red blood cell production in uremia. Arch Int. Med. 126:812-815, 1970.

30a. Eschbach JW, Mladenovic J, Garcia JF, et al. The anemia of chronic renal failure in sheep. J. Clin. Invest. 74:434-441, 1984.

31. Eschbach JW, Egrie JC, Downing MR, et al. Correction of the anemia of end-stage renal disease with recombinant human erythropoietin. N. Engl. J. Med. 316:73-78, 1987.

32. Eschbach JW, Adamson JW. Guidelines for recombinant human erythropoietin therapy. Am. J. Kidney Dis. 14:2-8, 1989.

33. Eschbach JW. Hematological problems of dialysis patients. In: Replacement of renal function by dialysis. JF Maher ed. Kluwer Academic Publishers, pp. 851-864, 1989.

34. Eschbach JW, Abdulhadi MH, Browne JK, et al. Recombinant human erythropoietin in anemic patients with end-stage renal disease. Ann. Int. Med. 111:992-1000, 1989.

35. Eschbach JW. The anemia of chronic renal failure: Pathophysiology and the effects of recombinant erythropoietin. Kidney Int. 35:134-148, 1989.

36. Fisher JW. Mechanism of the anemia of chronic renal failure. Nephron 25:106-111, 1980.

37. Fisher JW, Nelson PK, Beckman B, Burdowski A. Kidney control of erythropoietin (Chapter five). In: Renal endocrinology, MJ

Dunn ed. Williams & Wilkins, pp. 142-180, 1983.

38. Flaharty KK, Grimm AM, Vlasses PH. Epoetin: Human recombinant erythropoietin. Clinical Pharmacy 8:769-782, 1989.

39. Freedman MH, Saunders EF, Cattran DC, Rabin EZ. Ribonuclease inhibition of erythropoiesis in anemia of uremia. Am. J. Kidney Dis. II:530-533, 1983.

40. Frenken LA, Verberckmoes R, Michielsen P, Koene RA. Efficacy and tolerance of treatment with recombinant-human erythropoietin in chronic renal failure (pre-dialysis) patients. Nephrol. Dial. Transplant 4:782-786, 1989.

41. Fritschka E, Neumayer HH, Seddighi S. Effect of erythropoietin on sympathetic tone in chronic hemodialysis patients. Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program, p. 180A, 1989.

42. Garcia DL, Anderson A, Rennke HG, Brenner BM. Anemia lessens and its prevention with recombinant human erythropoietin worsens glomerular injury and hypertension in rats with reduced renal mass. PNAS 85:6142-6146, 1988.

43. Goldberg MA, Glass GA, Cunningham JM, Bunn HF. The regulated expression of erythropoietin by two human hepatoma cell lines. PNAS 84:7972-7976, 1987.

44. Goldberg MA, Dunning SP, Bunn HF. Regulation of the erythropoietin gene: Evidence that the oxygen sensor is a heme protein. Science 242:1412-1415, 1988.

45. Grimm AM, Flaharty KK, Hopkins LE, et al. Economics of epoetin therapy. Clinical Pharmacy 8:807-810, 1989.

46. Grutzmacher P, Ehmer B, Messinger, Scigalla P. Response to recombinant erythropoietin in aluminum overload and hyperparathyroidism. Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program, p. 111A, 1989.

47. Hakim RM, Zaoui P, Stone W. Cellular and soluble interleukin-2 receptors (IL2R) in uremia lymphocytes: Comparison between complement activating (CA) and non-complement activating membranes. Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program, p. 111A, 1989.

48. Haley NR, Adamson JW, Schneider GL, Eschbach JW. There are no uremic inhibitors to erythropoietin (EPO) in chronic renal failure (CRF). Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program, p. 319A, 1989.

49. Hampers CL, Streiff R, Nathan, DG, et al. Megaloblastic

hematopoiesis in uremia and in patients on long-term hemodialysis. N. Engl. J. Med. 276:551-554, 1967.

50. Hollomby DJ, Muirhead N, Hodsman AB, et al. The role of aluminum (Al) and PTH in erythropoietin (EPO) resistance in hemodialysis patients. Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program, p. 113A, 1989.

51. Hori K, Kumagai H, Onoyama K, et al. Effects of erythropoietin on anemia and hemodynamics in chronic renal failures without dialysis treatment. Am. Soc. Nephrology 21st Annual meeting, Abstracts on Recombinant Erythropoietin p. 5, 1988.

52. Jacobs K, Shoemaker C, Rudersdorf R, et al. Isolation and characterization of genomic and cDNA clones of human erythropoietin. Nature 313:806-810, 1985.

53. Jacobson LO, Goldwasser E, Fried W, Plzak L. Role of kidney in erythropoiesis. Nature 179:633-634, 1957.

54. Jamgotchian N, Hu MS, Abdella P. et al. Recombinant human erythropoietin (rHuEPO)-induced hypertension (HPT): Development of an experimental model. Am. Soc. Nephrology 21st Annual meeting, Abstracts on Recombinant Erythropoietin p. 18, 1988.

54a. Kaehny WD, Miller BE, White WL. Relationship between dialyzer reuse and the presence of anti-N-like antibodies in chronic hemodialysis patients. Kidney Int. 12:59, 1977.

55. Keane WF, Maddy MF. Host defenses and infectious complications in maintenance hemodialysis patients. In: Replacement of renal function by dialysis. JF Maher ed. Kluwer Academic Publishers, pp. 865-880, 1989.

56. Korbet SM. Comparison of hemodialysis and peritoneal dialysis in the management of anemia related to chronic renal disease. Seminars in Nephrology 9(Suppl. 1):9-15, 1989.

57. Koury ST, Bondurant MC, Koury MJ. Localization of erythropoietin synthesizing cells in murine kidneys by in situ hybridization. Blood 71:524-527, 1988.

58. Kushner D, Beckman B, Nguyen L, et al. The role of ployamines and uremic serum inhibitors in the anemia of end stage renal disease (ESRD). Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program, p. 52A, 1989.

59. Lacombe C, Da Silva J-L, Bruneval P, et al. Peritubular cells are the site of erythropoietin synthesis in the murine hypoxic kidney. J. Clin. Invest. 81:620-623, 1988.

60. Law ML, Cai G-Y, Lin F-K, et al. Chromosomal assignment of the human erythropoietin gene and its DNA polymorphism. PNAS 83:6920-6924, 1986.

61. Lim VS, Kirchner PT, Fangman J, et al. The safety and the efficacy of maintenance therapy of recombinant human erythropoietin in patients with renal insufficiency. Am. J. Kidney Dis. 14:496-506, 1989.

62. Lim VS, Fangman J, Flanigan MJ, et al. Effect of recombinant human erythropoietin on renal function in humans. Kidney Int. 37:131-136, 1990.

63. Lim VS, Fangman J, Flanigan M. The effect of recombinant human erythropoietin (r-HuEPO) on renal function in man. Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program p. 91A, 1989.

64. Lin F-K, Suggs S, Lin C-H, et al. Cloning and expression of the human erythropoietin gene. PNAS 18:7580-7584, 1985.

65. Loge JP, Lange RD, Moore CV. Characterization of the anemia associated with chronic renal insufficiency. Am. J. Med. 24:4-18, 1958.

66. Losekann A, Urena P, Casadevall N, Drueke T. aluminum (Al)induced resistance to erythropoietin (EPO) in rats. Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program, p. 120A, 1989.

67. Ludwig H, Fritz E, Kotzmann H, et al. Erythropoietin treatment of anemia associated with multiple myeloma. N. Engl. J. Med. 322:1693-1699, 1990.

68. Lundin AP. Quality of life: Subjective and objective improvements with recombinant human erythropoietin therapy. Seminars in Nephrology 9(Suppl 1):22-29, 1989.

69. Manzler AD, Schreiner AW. Copper-induced acute hemolytic anemia. Ann. Intern Med. 73:409-412, 1970.

70. Markson JL, Rennie JB. The anaemia of chronic renal insufficiency. Scot. Med. J. 1:320-322, 1956.

70a. Martin J, Moncada S. Blood pressure, erythropoietin, and nitric oxide. Lancet 1:644, 1988.

71. Maxwell P, Lappin T, MacManus M. Erythropoietin synthesis localized to hypoxic renal tubular cells. Kidney Int. 37:186A, abst. 64, 1990.

72. McDermott FT, Galbraith AJ, Corlett RJ. Inhibition of cell

proliferation in renal failure and its significance to the uraemic syndrome: A review. Scot. Med. J. 20:317-327, 1975.

73. McDonald JD, Lin F-K, Goldwasser E. Cloning, sequencing, and evolutionary analysis of the mouse erythropoietin gene. Mol. Cell. Biol. 6:842-848, 1986.

74. McGonigle RJS, Wallin JD, Shadduck RK, Fisher JW. Erythropoietin deficiency and inhibition of erythropoiesis in renal insufficiency. Kidney Int. 25:437-444, 1984.

75. Meytes D, Bogin E, Ma A, et al. Effect of parathyroid hormone on erythropoiesis. J. Clin. Invest. 67:1263-1269, 1981.

76. Miller CB, Jones RJ, Piantadois S. et al. Decreased erythropoietin response in patients with the anemia of cancer. N. Engl. J. Med. 322:1689-1692, 1990.

77. Miyake T, Kung CK-H, Goldwasser, E. Purification of human erythropoietin. J. Biol. Chem. 252:5558-5564, 1977.

78. Mohini R. Clinical efficacy of recombinant human erythropoietin in hemodialysis patients. Seminars in Nephrology 9(Suppl 1):16-21, 1989.

79. Neff MS, Kim KE, Persoff M, et al. Hemodynamics of uremic anemia. Circulation 43:876-883, 1971.

80. Nissenson AR, Marsh JT, Brown WS. et al. Brain function improves in chronic hemodialysis (CHD) patients (pts) after recombinant erythropoietin (rEPO). Am. Soc. Nephrology 21st Annual meeting, Abstracts on Recombinant Erythropoietin p. 13, 1988.

81. Nissenson AR. Recombinant human erythropoietin: Impact on brain and cognitive function, exercise tolerance, sexual potency, and quality of life. Seminars in Nephrology 9(Suppl 2):25-31, 1989.

82. Nonnast-Daniel B, Schaffer J, Frei U. Hemodynamics in hemodialysis patients treated with recombinant human erythropoietin. In: Erythropoietin: From Molecular Structure to Clinical Application. Baldamus CA, et al. eds. Contrib. Nephrol. Basel, Karger, vol. 76, pp. 283-291, 1989.

82a. Paganini EP. Overview of anemia associated with chronic renal disease. Seminars in Nephrology 9:3-8, 1989.

83. Podjarny E, Rathaus M, Korzets Z, et al. Is anemia of chronic renal failure related to secondary hyperparathyroidism? Arch Int. Med. 141:453-455, 1981.

84. Ponticelli C, Casati S. Correction of anaemia with recombinant human erythropoietin. Nephron 52:201-208, 1989.

85. Powell JS, Berkner KL, Lebo RV, Adamson, JW. Human erythropoietin gene: High level expression in stably transfected mammalian cells and chromosome localization. PNAS 83:6465-6469, 1986.

86. Quinn P, Acchiardo S, Cockrell S, et al. Response to erythropoietin (EPO) in patients (pts) on conventional (CD) and high flux dialysis (HF). Am. Soc. Nephrology 21st Annual meeting, Abstracts on Recombinant Erythropoietin p. 14, 1988.

87. Radtke HW, Claussner A, Erbes PM, et al. Serum erythropoietin concentration in chronic renal failure: Relationship to degree of anemia and excretory renal function. Blood 54:877-884, 1979.

88. Radtke HW, Frei U, Erbes PM, et al. Improving anemia by hemodialysis: Effect on serum erythropoietin. Kidney Int. 17:382-387, 1980.

89. Radtke HW, Rege AB, LaMarche MB, et al. Identification of spermine as an inhibitor of erythropoiesis in patients with chronic renal failure. J. Clin. Invest. 67:1623-1629, 1981.

90. Reissmann KR. Studies on the mechanism of erythropoietic stimulation in parabiotic rats during hypoxia. Blood 5:372-380, 1950.

91. Rege AB, Brookins J, Fisher JW. A radioimmunoassay for erythropoietin: serum levels in normal human subjects and patients with hemopoietic disorders. J. Lab. Clin. Med. 100:829-843, 1982.

92. Robertson RT, Haley RN, Guthrie M, et al. Recombinant erthropoietin improves exercise capacity in anemic hemodialysis patients. Am. J. Kidney Dis. 15:325-332, 1990.

93. Schwartz AB, Mintz GS, Kim KE, et al. Recombinant human erythropoietin (rHuEPO) increases MAP, TPRI and systolic and diastolic dysfunction with increased impedance to LV ejection due to increased HCT and RBC mass in pts with CRF. Am. Soc. Nephrology 21st Annual meeting, Abstracts on Recombinant Erythropoietin p. 20, 1988.

94. Segal GM, Eschbach JW, Egrie JC, et al. The anemia of end-stage renal disease: Hematopoietic progenitor cell response. Kidney Int. 33:983-988, 1988.

95. Sheehan, R. Erythropoietin and erythropoiesis: Applications in clinical medicine. Medical Grand Round, Feb. 25, 1988.

96. Sheingold SH. Cost-benefit analysis of using recombinant human erythropoietin for the anemia of chronic renal failure. Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program, p. 332A, 1989.

97. Sherwood JB, Goldwasser E. A radioimmunoassay for erythropoietin. Blood, 54:885-893, 1979.

98. Sherwood JB, Carmichael LD, Goldwasser E. The heterogeneity of circulating human serum erythropoietin. Endocrinology 122:1472-1477, 1988.

99. Shinaberger JH, Miller JH, Gardner PW. Disadvantages and risks of normal hematocrit (HCT) hemodialysis (HD). Am. Soc. Nephrology 21st Annual meeting, Abstracts on Recombinant Erythropoietin p. 15, 1988.

100. Shinaberger JH, Miller JH, Gardner PW. Erythropoietin alert: Risks of high hematocrit hemodialysis. ASAIO Transactions 34:179-184, 1988.

101. Spivak JL. The mechanism of action of erythropoietin. Intl J. Cell Cloning 4:139-166, 1986.

102. Spivak JL. Erythropoietin: A brief review. Nephron 52:289-294, 1989.

103. Spragg BP, Bentley DP, Coles GA. Anaemia of chronic renal failure. Polyamines are not raised in uraemic serum. Nephron 38:65-66, 1984.

104. Stenzel KH, Cheigh JS, Sullivan JF, et al. Clinical effects of bilateral nephrectomy. Am. J. Med. 58:69-75, 1975.
Wallner SF, Ward HP, Vautrin R, et al. The anemia of chronic renal failure: In vitro response of bone marrow to erythropoietin (38931). Proc. Soc. Exp. Biol. Med. 149:939-944, 1975.

104a. Stivelman JC. Resistance to recombinant human erythropoietin therapy: A real clinical entity? Seminars in Nephrology 9:8-11, 1989.

105. Stone WJ, Graber SE, Krantz SB, et al. Treatment of the anemia of predialysis patients with recombinant human erythropoietin: A randomized, placebo-controlled trial. Am. J. Med. Sci. 296:171-179, 1988.

106. Teehan BP, Sigler MH, Brown JM, et al. Hematologic and physiologic studies during correction of anemia with recombinant human erythropoietin in predialysis patients. Transplantation Proceedings 21:63-66, 1989.

107. Temple RM, Eadington DW, Swainson CP, Winney, R. Seizure related to erythropoietin treatment in patients undergoing dialysis. Br. Med. J. 300:46, 1990.

108. Tomura S, Tachibana K, Nakamura Y, et al. Enhanced platelet function and coagulability during treatment with recombinant erythropoietin (rEPO) in patients undergoing hemodialysis. Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program, p. 133A, 1989.

109. Urena P, Zingraff J, Losekann A, et al. Rise of plasma erythropoietin (EPO) in uremic patients (pts) after parathyroidectomy (PTx). Am. Soc. Nephroloyg 22nd Annual Meeting, Abstract Program, p. 133A, 1989.

110. Vallance P, Benjamin N, Collier J. Erythropoietin, haemoglobin, and hypertensive crises. Lancet 2:1107, 1988.

111. Van Stone JC. Who should receive recombinant human erythropoietin? Seminars in Nephrology 9(Suppl 2):3-7, 1989.

112. Van Stone JC. Resistance to recombinant human erythropoietin therapy: A real clinical entity? Seminars in Nephrology 9(Suppl 2):8-11, 1989.

113. Van Wyck DB. Iron deficiency in patients with dialysis-associated anemia during erythropoietin replacement therapy: strategies for assessment and management. Seminars in Nephrology 9(Suppl 2):21-24, 1989.

114. Van Wyck DB. Iron management during recombinant human erythropoietin therapy. Am. J. Kidney Dis. 14(Suppl 1):9-13, 1989.

115. Wall AJ, Wong GY, Clemons GK, et al. Erythropoietin-hematocrit feedback circuit in the anemia of end-stage renal disease. Kidney Int. 31:1205-1209, 1987.

115a. Wolcott DL, Schweitzer S, Nissenson AR. Recombinant erythropoietin (eEPO) improves cognitive function (CF) and quality of life (QL) of chronic hemodialysis (CHD) patients (Pts). Am. Soc. Nephrology 21st Annual Meeting, Abstracts on Recombinant Erythropoietin, p. 16, 1988.

116. Wallner SF, Vautrin RM. Evidence that inhibition of erythropoiesis is important in the anemia of chronic renal failure. J. Lab. Clin. Med. 97:170-178, 1981.

117. Watson AJ. Adverse effects of therapy for the correction of anemia in hemodialysis patients. Seminar in Nephrology 9(Suppl 1):30-34, 1989.

118. Williams JD, MacDougall IC, Davies ME, et al. Recombinant erythropoietin (EPO) treatment is accompanied by a reduction on protein C and S levels. Am. Soc. Nephrology 22nd Annual Meeting, Abstract Program p. 193A, 1989.

119. Winearls CG, Oliver DO, Pippard MJ, et al. Effect of human erythropoietin derived from recombinant DNA on the anaemia of patients maintained by chronic haemodialysis. Lancet 2:1175-1177, 1986.

120. Yawata Y, Kjellstrand C, Buselmeier T, et al. Hemolysis in dialyzed patients: Tap water-induced red blood cell metabolic deficiency. Trans. Amer. Soc. Artif. Int. Organs 18:301-304, 1972.

121. Zaoui P, Bayle F, Maurizi J, et al. Early thrombosis in kidney grafted into patient treated with erythropoietin. Lancet 1:956, 1988.

122. Zingraff J, Drueke T, Marie P, et al. Anemia and secondary hyperparathyroidism. Arch Int. Med. 138:1650-1652, 1978.