The clinical pharmacology of biotechnology products
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ERYTHROPOIETIN.

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The frequent occurrence of anemia in patients with renal failure is well known since the first classical paper on kidney disease from Richard Bright in 1836. However, the role of the kidney in regulating erythropoiesis was not recognised until 1957 when Jacobson and coworkers showed that removal of kidneys nearly abolishes erythropoietin (EPO) production in rats (1).

Subsequent studies showed that the normal isolated perfused kidney synthesizes EPO when perfused with a serum free medium and that this synthesis is augmented when the kidney is perfused at low pO2 (2).

The kidney is responsible for sensing oxigen availability to tissues, as well as for releasing EPO into circulation. The site of EPO generation within the kidney is a matter of debate. Peritubular interstitial cells, mesangial cells and yuxtaglomerular apparatus have all been considered as possible sites of renal EPO generation (3). Hepatic generation of EPO contributes only 10 to 15 % of total EPO in normal adults.

FUNCTION OF ERYTHROPOIETIN

EPO induces bone marrow red cell formation by stimulating proliferation, downstream differentiation and maturation of erythroid progenitors and precursors. In high titers EPO causes early release of reticulocytes into circulation (4). The most primitive erythroid progenitor cell responding to EPO is the burst forming unit-erythroid (BFU-E), and the more mature, the colony forming unit-erythroid (CFU-E). Proliferation of BFU-E requires a specific growth factor generated by lymphocytes or monocytes, and BFU-E will die if further differentiation does not occur.

BFU-E are minimally responsive to EPO, and CFU-E is more sensitive and specific for EPO action. EPO binds to receptors on the CFU-E, stimulating the proliferation and differentiation of these cells into erythroblats (5, 6).

Some studies show the existence of two classes of binding sites on erythroid progenitor cells, a high affinity and a low affinity EPO receptor. The high affinity receptor seems to be essential in the response to EPO. It is unknown how the receptors modulate the action of EPO. However, in the early stage of in vitro growth CFU-E have an almost absolute need for EPO (7, 8).

Although this early need for EPO by CFU-E is almost absolute, the often large amount of EPO already internalized into the cell cannot satisfy this need. This implies that CFU-E require repeated occupancy of the rapidly turning over EPO receptors. Thus, a more or less continuous presence of EPO is necessary to maintain the development, maturation and differentiation of red cells. In vivo, the biological effect of a given dose of EPO is largest when it is administered intermittently in divided gifts. These studies have been made using recombinant human erythropoietin (r-HuEPO) and they might explain some of the complex therapeutic properties r-HuEPO exhibits.

The sequence of biochemical postreceptors events tiggered by EPO is still undefined. More is known about the later events that occur as the erythroid progenitor cells mature into late erythroblasts. At the cellular level EPO enhances Ca++ uptake into the cells. Total RNA synthesis increases due to the activation of transcription within 3 to 4 hours and does not apprear to require protein or DNA-synthesis. A marked hemoglobin synthesis follows within 12 hours.

PATHOGENESIS OF THE ANEMIA OF CHRONIC RENAL FAILURE (CRF).

The most important cause of the anemia of CRF is decreased EPO production from the diseased kidney, but other factors contribute to the pathogenesis of the anemia.

Decreased red cell survival.

Decreased red cell survival to approximately half normal is seen in patients with advanced renal insufficiency (9). However, hemolysis is mild enough that a normal hematopoietic system should be able to compensate for it. Hemolysis is caused by substances in uremic plasma that interfere with the red blood cells membrane's ability to effectively pump sodium from the cells.

Inhibition of erythropoiesis.

Several lines of evidence suggest that uremic toxins retained in patients with CRF interfere with bone marrow function (6, 9). Uremic sera

inhibit proliferation of BFU-E and CFU-E, hemo synthesis and bone marrow thymidine incorporation. Several compounds have been suggested to be the specific erythroid inhibitor including spermine, parathyroid hormone (PTH) and ribonuclease (9), but recent studies suggest that uremic inhibition of in vitro hematopoiesis is not specific.

Erythropoietin deficiency.

The anemia of CRF is primarily due to decreased EPO secretion by the diseased kidney since serum EPO levels are generally inappropriately low for the degree of anemia. With the purification of human EPO valid radioimmunoassays (RIA) for EPO were developed, and using these methods, mean values for serum EPO were 17.2 mU/ml for normal males, 18.8 mU/ml for normal females, 97 mU/ml for iron defficiency anemia patients, and greater than 1000 mU/ml for patients with anemia secondary to bone marrow failure (10). In our own experience, using the RIA we have found normal or high serum EPO levels in CRF patients, but they are low in relation to the degree of anemia of these patients. The usual inverse correlation between hematocrit or hemoglobin and serum EPO levels as would be expected if renal function were normal is not seen in patients with CRF.

Several studies have shown that in CRF patients serum EPO levels do not change with the decline in GFR despite the development of severe anemia (6). However, other authors recently showed a rise in serum EPO in hemodialysis patients in response to spontaneous hemorrhage, and suppression in serum EPO levels following blood transfusion (11).

The available evidence suggest that the hypoxia-EPO-hematocrit feedback system functions at a lower set point of tissue oxigenation in patients with renal failure than in normal individuals.

Other factors.

Blood loss may also contribute to the anemia in patients with advanced renal failure, as consequence of platelet dysfunction. Repetitive blood loss may cause iron deficiency.

Aluminium toxicity, hyperparathyroidism, folate deficiency, hypersplenism and acute hemolysis are aggravating factors of the anemia of CRF.

RECOMBINANT HUMAN ERYTHROPOIETIN.

Miyake and coworkers in 1977 isolated a large quantity of EPO from the urine of severely anemic patients and this material served as a source of pure EPO for the study of its chemical structure (12).

EPO is a glycoprotein with a molecular weight of approximately 34,000 daltons and contains approximately 25 % carbohydrate consisting mostly of sialic acid (6). Removal of the sialic acid portion of the molecule abolishes the in vivo but not the in vitro, activity of EPO. The carbohydrate moiety is not critical for the erythropoietic action of EPO, but prevents its rapid clearance.

The protein is made up of 166 aminoacids. Part of the aminoacid sequence was used to predict the base composition of a corresponding cDNA, wich in turn was used as a probe to identify the entire EPO gene. The EPO gene is encoded as a single copy on chromosome 7.

Molecular biologists have isolated the gene and inserted it into mammalian cells capable of synthesizing unlimited quantities of EPO. It was necessary to use animal cells rather than bacteria because EPO is highly glycosylated, and only animal cells provide the necessary sugar components.

The biological activity and immunological properties of human purified EPO and recombinant human EPO have not been distinguishable.

The first published reports of the successful isolation, cloning and expression of the human EPO gene appeared in 1985 (13, 14), and large amounts of r-HuEPO became available for clinical trials. The results of these trials involving anemic patients on hemodialysis from the basis of multicenter trials in the United States, Western Europe and Japan, coupled with the results in anemic patients with CRF not yet on dialysis, support the concept that EPO deficiency is the major mechanism responsible for the anemia (15, 16).

These studies showed that intravenous EPO therapy could fully reverse the anemia of CRF, and that there was a dose-dependent rate of response to EPO.

THERAPEUTIC USE OF r-HuEPO.

Large multicenter trials have extended and confirmed the initial observations with r-HuEPO. Virtually all patients treated responded appropiately to a intravenous dose of 50 to 300 U/kg, three times a week and increased their hematocrit to the target range. The patients no longer needed transfusions and their quality of life improved. Lower doses and differents routes of administration have been explored with similar good results.

Which patients should be treated?

Patients who are symptomatic of anemia should be treated. But it may be difficult to define who is symptomatic. Patients requiring blood transfusions should be treated. In the non-transfusion dependent patient, the following symptoms are associated with anemia and improve with partial correction of the anemia: physical fatigue, poor appetite, coldness, disordered sleep/awake pattern, depression, sexual desinterest and mental slowness. These symptoms are often present with a hemoglobin < 8 g/dl. However, dialysis patients with symptoms of ischemic heart disease, even if their hemoglobin is above 8 g/dl, sould also be considered for EPO therapy.

Patients who have recently commenced dialysis may experience spontaneous amelioration of their anemia to more acceptable levels, thus negating the need for EPO.

For patients with CRF not yet requiring dialysis the situation is even less clear. Few of these patients have a hemoglobin level < 8 g/dl and if they are symptomatic, EPO therapy may be appropiate. The increased blood viscosity resulting from the improved hematocrit may adversely affect renal perfusion and this accelerate the decline in renal function, although the clinical studies to date do not support this hypothesis (17, 18).

In all patients, any other treatable cause of anemia must be excluded before starting EPO therapy.

Dosage of EPO and routes of administration.

A variety of dosage regimes and routes of administration have been employed. The greatest experience is with intravenous therapy in hemodialysis patients and the earliest studies showed a dose-dependent rate of response to EPO. However, the risk of side effects such as severe hypertension and thrombotic complications is lessened with a hemoglobin rise not exceeding 1 g/dl/month.

As consequence the recommended starting dose of EPO has declined in comparison with earlier studies. Most centers now use an initial intravenous dose in the range of 40-50 U/kg three times a week, for hemodialysis patients. A similar IV dosage regime has been used with good results in patients not yet on dialysis (17).

The IV route is impractical for regular use in CAPD patients. Obvious alternatives to be considered include the intraperitoneal and subcutaneous routes. An effective clinical response has been obtained in

these patients with a dose of 300 U/kg/week using the intraperitoneal route, but a similar response has been obtained with only 120/U/kg/week when EPO is given subcutaneously (17).

In the other hand, it has been shown that a 50 % reduction in dose can be achieved at optimal hemoglobin level by switching from IV to SC administration, in hemodialysis patients (19).

Thus, the SC route appears to be gaining popularity, not only in CAPD patients but also in hemodialysis and pre-dialysis patients, and evidence to date suggest that lower doses of EPO may be used when given by this route (60-150 U/kg/week). If the patient can be taught to give their own SC injection without stress or discomfort, then the daily dosing regime may be worth considering (14 U/kg/day) (20).

Pharmacokinetics.

Following IV administration of EPO, plasma concentrations reach a high peak shortly after the injection, with a mean T 1/2 ranging from 4.9 to 9.3 hours (7, 21). In most studies, the apparent volume of distribution equaled the plasma volume. The liver is considered the most likely site of degradation of EPO, but the bone marrow may make a contribution to EPO degradation.

After SC administration plasma concentrations of EPO start to increase after 2 hours. Peak concentrations are found at 12 to 18 hours and are much lower when compared to IV administration, but they remain above baseline for up to 72 hours (7, 21).

Target hemoglobin and rate of rise.

It is possible to fully correct the anemia of CRF with EPO. However, in comparing the benefits with the risks, partial correction of the anemia seems the best compromise. A linear increase in the hematocrit leads to an exponential rise in whole blood viscosity, which in turn is thought to contribute to many of the side effects of EPO therapy, such as hypertension and thrombotic complications.

The optimum hemoglobin seems to be in the range of 10-12 g/dl. Nevertheless, this is a very arbitrary guideline, and some flexibility is necessary in treating individual patients. Since the main aim of EPO therapy is to reverse the symptoms of anemia, differing thresholds at which this occurs may influence the appropriate target hemoglobin.

With regard to the rate of rise of the hemoglobin response, an increase of 1 g/dl/month should not be exceeded.

THERAPEUTIC EFFECTS OF EPO.

Correction of anemia in CRF patients results in better tissue oxigenation. The clinical benefits of this improved oxigenation include improved exercise tolerance, skin circulation and central nervous system function.

Tha almost constant feeling of tiredness is relieved, so that most patients are able to increase their activities, either in their work or their social life. There are also reports of increased appetite, relief of Raynauds' phenomenon, improvement of angina and of increased libido.

Exercise tolerance has been examined by several groups, showing a significant improvement. Working capacity, maximal oxigen consumption and anaerobic threshold increase after EPO therapy. A significant progresive reduction in left ventricular mass, measured using echocardiography, and a consequent improvement in cardiac function has been noted by different authors (17).

A number of investigators using questionnaires have now provided evidence of improvement of quality of life. An improvement in cognitive function with EPO therapy has been suggested. Improvement in sexual performance after 4 months of EPO therapy has been related with normalization of prolactin levels in these patients (22).

The effect of EPO on iron metabolism.

With the response to EPO, erythropoiesis may be stimulated as much as 3 to 4 times normal. This imposes a demand to movilize iron from reticuloendothelial storage sites and to make it available to transferrin for transport to the marrow for incorporation into hemoglobin. Severely anemic individuals who begin therapy with EPO are at risk of becoming iron deficient during the course of therapy. Currently, recommendations for managing patients during the induction phase of therapy include oral iron supplements, and in some patients IV administration of iron dextran.

In some patients the response to EPO is so brisk that movilization of iron from storage sites cannot keep place with the demand. In this setting, EPO may become less efective despite the fact that serum ferritin values clearly indicate that adequate iron stores are present. This condition represents a "functional" iron deficiency. The dose of EPO may be reduced or supplemental iron may be given orally or intravenously.

ADVERSE EFFECTS

The major adverse effect of raising hematocrit is aggravation of pre-existing hypertension, related to changes in peripheral vascular resistance Peripheral vascular resistance increases as consequence of the increase of whole blood viscosity and by relief of hypoxia-induced peripheral vasodilation.

Although there is usually a normalisation of the elevated cardiac output as the anemia is corrected, occasionally this does nor occur, and a sustained high cardiac output would result in an increase in blood pressure (17).

A further major complication of EPO therapy is thrombosis of the arteriovenous fistula. This tendency for EPO treatment to increase the risk of thrombosis suggested that it may have a beneficial effect on the bleeding diathesis associated with uremia.

Less frequent side effects are flu-like symptoms,fever, bone pain, myalgia and seizures.

Table I shows the frequency of different adverse EPO effects from an European Multicenter Study (23), in 150 patients with a follow-up of one year. Adverse effects were arbitrarily classified as related to the hematocrit increse, to the drug itself and as concomitant events.

T A B L E I

EPO ADVERSE DRUG EFFECTS

1.- Related to the hematocrit increase:	
Hypertension	32 %
Clotting	12 %
Thrombosis of the fistula	14.7 %
Weight gain	10 %
Hyperkaliemia	7 3 %
2.- Related to the drug:	
Musculoskeletal pain	30 %
Headache	26.6 %
Pyrexia	13.3 %
Flu-like syndrome	6 6 %
Pruritus	12 %
Rash	2 6 %
3.- Concomitant events:	
Seizures	1 9 %
Cerebrovascular accidents	1.3 %
Myocardial infarction	1.3 %
Sudden death	0.6 %

Most of the adverse effects seems to be related to the hematocrit increase. In consequence, the aim of the treatment should be to increase the hamatocrit gradually by the use of an appropriate dosage regime of EPO with a view to reducing their incidence.

Genuine intolerance to EPO sufficient to warrant stopping the hormone is absolutely rare. There have been no reports of antibody formation.

EPO RESISTANCE

The large multicenter trials of EPO indicate that 95-98 % of patients treated with EPO will respond.

Potential causes of EPO resistance are iron deficiency, B12/folate deficiency, aluminium toxicity, hyperparathyroidism, infection, malignancy, blood loss and hemolysis.

Thus, patients with a poor response to EPO , or loss of a previous response, require investigation for an underlying cause.

NEW INDICATIONS

The efficacy of EPO is now being evaluated in non renal causes of anemia, such as rheumatoid arthritis, malignancy, thalassemia, sickle cell disease, myelofibrosis, etc. Preliminary reports show the efficacy of EPO therapy in anemic AIDS patients treated with AZT.

CONCLUSION

EPO replacement therapy wiyh r-HuEPO has been one of the most dramatic medical advances and the most clinically important achievement of the last decade in the treatment of chronic renal failure patients. EPO therapy demonstrates that the primary cause of anemia of uremia is a deficiency in renal EPO and that many symptoms of uremic syndrome are consequence of the associated anemia. Substancial reversal of anemic symptoms and quality of life improvement is possible when anemia is corrected with EPO therapy.

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Due to unavoidable circumstances, the discussion after this presentation could not take place.