Recombinant-human erythropoietin and the effects of different routes of administration

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Introduction

In this report we will discuss the different routes of administration of recombinant-human erythropoietin (r-HuEPO). For better understanding, some aspects of the pharmacodynamics and pharmacokinetics of r-HuEPO will be pointed out. The consequences for practical therapy will be summarized.

Pharmacodynamics

Erythropoietin (EPO) plays an essential role in the regulation of red blood cell development [1]. Conclusive evidence exists that the kidney is the major source of EPO [2]. Our understanding of the link between reduced oxygen supply to the tissues and the synthesis of EPO in the kidney is still poor, whereas the exact mechanisms for the control of EPO production are also unknown. EPO binds to receptors on the erythroid colony-forming units (CFU-E) [3]. It serves as a cellular growth factor, stimulating the proliferation and differentiation of these cells into erythroblasts. It also acts on late erythroid burst-forming units (BFU-E), which differentiate into CFU-E. EPO may also promote the final maturation to reticulocytes and the release of reticulocytes into the circulation. Thus, whether an erythroid progenitor cell responds to EPO by proliferation or differentiation depends on its level of maturation. Erythroid progenitor cells respond to a variety of growth and development promoting factors in their micro-environment, but only EPO is obligatory in vivo for terminal differentiation. The mechanisms of action of EPO on these cells are also still largely unknown.

BFU-E and CFU-E are present in the bone marrow at a very low frequency of 0.1 to 1.0 %. This explains why studies of EPO receptors using normal haematopoietic tissues are difficult to perform. However, homogeneous populations of enriched murine progenitor cells that respond to EPO could be generated by infection of mice with «Friend virus that produces anaemia» (FVA). In addition, some erythroleukemia cell lines showed a growth dependency on EPO in culture. Cross-linking studies with ^{125}I-r-HuEPO involving these cell populations have shown that EPO combines with specific binding sites on the cell surface in a typical hormone-receptor interaction [3]. Other human cells that show binding of EPO, although to only a small extent, are certain cells in the placenta. 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. In vitro, purified murine CFU-E show a rapid turnover of the high affinity receptors [4]. Low affinity sites persist for a much longer time and at a larger number. 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.

Erythroblasts can grow in the presence of much lower amounts of EPO. Although the 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 in in vitro culture systems to maintain the development, maturation and differentiation of red cells. This is also in keeping with results of the in vivo bioassay of EPO where the biological effect of a given dose EPO was largest when it was administered intermittently in divided gifts. These findings might explain some of the complex therapeutic properties r-HuEPO exhibits. They will be discussed later in this article.

The detailed initial sequence of biochemical postreceptor signaling events triggered by EPO is still undefined. Much has to be learned about the EPO-EPO receptor interactions and the kinetics of the EPO receptor. These processes will probably be better understood when the EPO receptor will have been

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isolated, purified and characterized. Many studies are now addressing these questions.

More is known about the later events that occur as the erythroid progenitor cells mature into late erythroblasts [1]. At the cellular level EPO enhances Ca^{2+}-uptake into the cells within one minute and the hormone is present in the cell by 10 minutes. However, no direct evidence of internalization of EPO or the EPO-EPO receptor-complex exists. Total RNA-synthesis increases due to the activation of transcription within three to four hours and does not appear to require protein or DNA-synthesis. After six hours α and β globin gene transcription rate increases, with a concomitant increase in the number of transferrin receptors. A marked haemoglobin synthesis follows within 12 hours.

Pharmacodynamics

To produce its characteristic effects a drug must be present in appropriate concentrations at its sites of action. Although obviously a function of the amount of drug administered, the concentrations attained also depend upon the extent and rate of its absorption, distribution, biotransformation and elimination. The pharmacokinetics of single doses of r-HuEPO have been studied in small groups of patients on haemodialysis and on CAPD, and in pre-dialysis patients and healthy volunteers. In these studies the intravenous (IV), subcutaneous (SC) and intraperitoneal routes of administration were investigated.

After IV injection, r-HuEPO rapidly distributes throughout the plasma compartment. In most studies the apparent volume of distribution equaled the plasma volume. Distribution studies in animals reveal that most uptake of r-HuEPO occurs in the liver, kidney, and spleen. However, specific uptake occurs mainly in the bone marrow [5].

The specific pathways in the metabolic clearance of r-HuEPO have not yet been identified. The liver is considered the most likely site of degradation of r-HuEPO, but the bone marrow may make a significant contribution to r-HuEPO degradation. The precise size of the contribution of the bone marrow to the clearance of r-HuEPO at physiological plasma levels will remain uncertain until equilibrium studies, measuring the total turnover of r-HuEPO in the bone marrow, will have been done.

Following IV administration of r-HuEPO, plasma concentrations reach a high peak shortly after the injection. The elimination half-life of IV r-HuEPO ranged between 6 and 12 hours (mean 9.3 ± 3.2) in 11 haemodialysis patients [6]. This was comparable with the mean plasma half-life of r-HuEPO in CAPD patients, being 8.2 hours (range 6.2 to 10.2) [7]. In another study the elimination half-life of IV r-HuEPO ranged from 2.3 to 7.3 hours [8]. These different results can be explained by the great interindividual variations of r-HuEPO elimination together with the small numbers of patients that have been investigated. However, small sifferences between the r-HuEPO molecules of the various commercial preparations are also likely to result in differences in pharmacokinetic profile. Some investigators noted a decrease in half-life after multiple IV injections [9]. In haemodialysis patients treated with 500 U/kg IV the half life decreased from 9.3 hours to 6.2 hours after 7 injections in 5 of 6 patients [6]. Similar effects were seen in pre-dialysis patients [10]. On the first day of therapy the half-life was 7.7 hours, whereas on day 54 it had decreased to 4.6 hours. The increase in plasma clearance can possibly be explained by an increased consumption of r-HuEPO by the bone marrow once the erythroid cells have started to proliferate during the correction phase. However, other investigators reported no change in elimination half-life [8].

After SC administration of r-HuEPO, plasma concentrations start to increase after two hours [7-9, 11]. Peak concentrations are found at 12 to 18 hours. They are maintained at a plateau level for at least the next 12 to 16 hours. Peak concentrations are of course much lower when compared to IV administration. In one study the mean relative bioavailability (AUC) of SC r-HuEPO was found to be 21.5 % (range 11.3-36.0 %) [7], whereas others found 49 % (range 23-79 %) [11].

With IP administration to CAPD patients, increased concentrations of r-HuEPO are detectable within one to two hours [7, 8, 11]. Plasma r-HuEPO values do not reach steady state levels due to the relatively short dwell times and the removal of r-HuEPO from the peritoneal cavity after the dwell. However, in many patients r-HuEPO absorption into the blood stream continued for several hours after rinsing of the peritoneal cavity, suggesting pooling of the hormone in the lymphatic system. The mean relative bioavailability of r-HuEPO after IP administration was 2.9 % (range 1.2-6.8 %) [7], and 6.8 % (range 2.2-12 %) in another study [11]. However, r-HuEPO absorption from the peritoneal cavity increases when the concentration of r-HuEPO in the dialysis fluid is increased by using smaller volumes of dialysis fluid. Also a long dwell time increases the absorption. Thus, the results of the calculated AUC after IP administration strongly depend on the dialysis scheme used. Preliminary data from IP administration of r-HuEPO in a small volume of dialysis fluid in children treated with Intermittent Peritoneal Dialysis show a bioavailability that is comparable to SC administration.

Therapeutic use

Large multicentre trials have extended and

confirmed the initial observations with r-HuEPO. Virtually all patients treated responded appropriately to r-HuEPO (50 to 300 U/kg IV, three times a week) and increased their haematocrit to the target range. The patients no longer needed transfusions and their quality of life improved. Patients have now been receiving r-HuEPO for up to three years. Resistance to r-HuEPO has not been observed neither have antibodies to the hormone been detected.

The most important adverse effect with all routes of administration is hypertension. Preliminary analysis of the US and European experiences with r-HuEPO indicates that the development of hypertension is not significantly related to the dose of r-HuEPO nor to the rate of increase of the haematocrit. However, those patients with more severe anaemia (haematocrit < 20 %) are at larger risk for the development of hypertension. This hypertensive effect of r-HuEPO treatment is moderate or absent in hypotensive or normotensive patients, but is striking in patients with pre-existing hypertension. The reported incidence of hypertensive complications is about 30 % [12]. Hypertension is absent in anaemic patients without renal disease treated with r-HuEPO. This indicates that r-HuEPO by itself does not cause hypertension. The appearance of hypertension is probably related to the haemodynamic changes induced by correction of the anaemia [13]. In haemodialysis patients there is a peripheral vasodilation induced by the anaemia. When the haematocrit increases during r-HuEPO-treatment, but also by blood transfusions, the peripheral vasodilation decreases and the peripheral resistance increases. The cardiac output decreases and diastolic blood pressure increases concomitantly. During r-HuEPO treatment whole blood viscosity increases, and this can also increase peripheral resistance. If the subsequent haemodynamic adaptations are not adequate or occur to slowly, peripheral resistance will be inappropriately increased with respect to cardiac output, thereby producing hypertension. Once the patients have been adjusted to their new haemodynamic status the risk of hypertensive complications is reduced. A slow increase in haematocrit allows time for these haemodynamic adaptations, and for appropriate blood pressure control. Expansion of the extracellular volume is the most important mechanism in the cause of the elevated blood pressure in haemodialysis patients. Since haemodialysis patients are unable to regulate their own extracellular volume, blood pressure should carefully be controlled by appropriate dialysis ultrafiltration and with antihypertensive medication.

Intravenous administration

In the first study in haemodialysis patients a dose finding protocol was used [14]. IV r-HuEPO 15 U/kg three times a week was the minimum effective dosage. However, the rise in haematocrit was relatively slow. At a dosage of 50 U/kg IV three times a week, reticulocyte count and haematocrit often increase within one to three weeks after the start of the therapy. Most patients no longer require transfusions after this time. The response to r-HuEPO showed a dose-dependency. Target haematocrit is achieved within four to six weeks with higher dosage regimens (up to 500 U/kg three times a week have been given IV) depending on the initial degree of anaemia. In the bioassay for EPO the production of red cells is a function of the logarithm of the dose. This implies that doubling the response will only be possible at the expense of very large doses of r-HuEPO. Apart from the cost-effectiveness questions that are raised by the use of high dosages, lower dosages of r-HuEPO are to be preferred, because a slow increase in haematocrit causes less side effects. Clinical trials have shown that most patients require between 25 and 125 U/kg three times per week to maintain a stable haematocrit. A minority (± 25 %) of patients require 150 to 300 U/kg three times per week. Some patients will not respond to the 50 U/kg dose.

The «Statement on the Clinical Use of Recombinant Erythropoietin in Anemia of End Stage Renal Disease» by the «Ad Hoc Committee for the United States National Kidney Foundation» recommends administration of r-HuEPO IV three times weekly at a dose of 150 U/kg until the haematocrit reaches 30 % [15]. The dose can then be decreased to 75 U/kg and should be adjusted up or down in amounts of 12.5 to 25 U/kg until a dose is achieved that will maintain the haematocrit at about 35 %. However, in patients with unstable hypertension, they advise to use a lower initial dose (50 to 100 U/kg). These recommendations involve a rather high initial dose of r-HuEPO. However, patients with relatively high haematocrits will not need high initial doses of r-HuEPO because they will reach and pass the 30 % level in a very short time. Moreover, in patients with low haematocrit and patients with a history of hypertension (more than 30 %) high initial doses are hazardous. Since most of the remaining patients will respond to a low initial dose, a «low and slow» dosing protocol is in common use in Europe. Emphasis is placed on dose titration to achieve target haematocrit values. The recommended starting dose is 50 to 75 U/kg three times a week IV, adjusted by increments of 25 U/kg at six weekly intervals, to achieve target haematocrit valus within 12 to 18 weeks.

Subcutaneous administration

Bommer studied patients who had been receiving intermittent IV r-HuEPO three times a week for more than one year and then received r-HuEPO SC three

Table I. Comparison of the response to IP and SC r-HuEPO in CAPD-patients

Week	IP r-HuEPO			SC r-HuEPO		
	Ht.[a] (%)	Dose (U/kg)	n	Ht.[a] (%)	Dose (U/kg)	n
0	19 ± 4	101 ± 4	10	22 ± 1	100 ± 2	8
4	23 ± 6	101 ± 4	10	32 ± 6	88 ± 35	8
9	28 ± 6	100 ± 10	10	35 ± 5	88 ± 68	8
13	30 ± 7	94 ± 17	10	38 ± 3	62 ± 41	6
21	32 ± 6	84 ± 54	10	37 ± 4	34 ± 38	6

Results are expressed as mean ± SD.
Ht.[a] = haematocrit.

times a week [16]. In this first study, the SC dose could be reduced to 50 % of the IV dose without a decrease in the haenmoglobin level. In a larger follow-up study he showed that the dose reduction was maintained at about 40 %. With SC r-HuEPO the relative bioavailability is only 20-50 % compared to IV. However, it results in a more prolonged plasma half-life and consequently a longer persistence of adequate plasma r-HuEPO values. The difference in pharmacokinetic profiles seen after IV compared with SC administration is reflected in the increased clinical efficacy of this treatment modality. The before-mentioned studies on receptor kinetics may explain this phenomenon. Thus, moderate continuing stimulation following SC injection is more effective than pulsatile stimulation after IV injection. Generally, the starting dose with SC administration should not exceed 50 U/kg, in order to avoid too rapid a rise in haematocrit. The incidence of hypertension appears to be the same with both SC and IV administration. The more acute adverse effects of IV r-HuEPO (such as «flu-like syndrome», headache, bone and muscle pains), seem to occur less frequently with SC administration. In a minority of patients the SC application of certain r-HuEPO preparations results in a more or less short-lasting burning pain. This might be the result of the properties of the buffered solution used or of the albumin preparation present in the solution. In these cases changing the preparation will relieve these local problems.

More studies involving larger numbers of patients are necessary to establish the benefits of SC dosing more precisely. However, the results already available suggest that the SC route for r-HuEPO is at least as effective as the IV route. The maintenance dose can probably be reduced considerably, resulting in large cost-savings. There are still not enough data on the effectiveness and dosage of r-HuEPO in treating patients undergoing CAPD. However, analysis of the current trials shows that the response to r-HuEPO in CAPD patients is not different from the response in haemodialysis and pre-dialysis patients. Moreover, SC dosing three times a week in CAPD patients will be far more convenient than IV dosing.

Intraperitoneal administration

IP administration of r-HuEPO is still under investigation. The relative bioavailability of r-HuEPO, is only 20-50 % after SC and less than 10 % after IP administration when compared to IV. These data might suggest that in particular IP r-HuEPO would not be effective. However, in spite of these low values the therapeutic efficacy of SC has already been proved. We investigated the therapeutic effectiveness of these treatment modalities. Patients on CAPD injected r-HuEPO (100 U/kg) SC (n = 8), or IP in their dialysis bag (n = 10) three times a week. With r-HuEPO added to the bag the dialysis volume was 1 litre and the glucose concentration was 1.5 %. The dwell times were 10 to 12 hours during the night. The results are shown in the table.

In the IP group the rate of increase in haematocrit was lower and the maintenance dose was larger. However, the results in the IP group were comparable to the results in IV treated pre-dialysis patients, who were matched for initial haematocrit. Four episodes of peritonitis occurred in the IP group and none in the SC group. The incidence of peritonitis was not increased when compared to the pre-treatment incidence. The incidence of hypertensive events was the same in both groups. Preliminary experience with IP administration in several children treated with CAPD shows even more favourable results. In particular children treated with Intermittent Peritoneal Dialysis have low dose requirements.

Dosing schedule

Information on the optimal dosing schedule and the best route of administration is still incomplete. IV injections three times a week at the and of dialysis are convenient in haemodialysis patients, but are not necessarily the most cost-effective way of administration. Dosing once weekly gives a sufficient response in most patients. However, Muirhead showed that this schedule requires larger doses than dosing three times a week [15]. Moreover, Bommer showed that when r-HuEPO was given twice weekly instead of three times, 30 % more r-HuEPO was needed [16]. More

frequent dosing than three times a week does not seem to add significant value. These observations suggest that a continuous minimum plama r-HuEPO level is required and this is in keeping with the earlier mentioned studies, demonstrating that repeated occupancy of the rapidly turning over r-HuEPO-receptors is required.

It is still unknown what the optimal target value for the haematocrit should be. In most patients increasing the haematocrit to 35 % improves considerably the quality of life, and in particular exercise tolerance. There are no studies showing significant further benefits if the haematocrit is increased to more than 35 %. Moreover, this might result in increased difficulty in controlling blood pressure or increased an incidence of thombotic complications in arteriovenous fistulae or clotting in the artificial kidney during haemodialysis.

Conclusions

In the last five years, the rapid development of modern biotechnological techniques made it possible to devise reliable assays for the determination of EPO, and to produce r-HuEPO on a large scale. Substitution therapy with r-HuEPO has been rapidly introduced in nephrology and its place as an effective method to correct the anaemia of chronic renal failure is firmly established. However, the mechanisms whereby EPO production in the kidney is regulated are still not unravelled. Moreover, the complex actions of r-HuEPO on the red blood cell-forming cells are incompletely understood. In various studies it has appeared that descriptive pharmacokinetics alone do not appropriately predict the biological response to r-HuEPO. This is illustrated by the aparently better effectiveness of SC r-HuEPO when compared to IV, despite the lower bioavailability of the former application.

Further studies to investigate the optimum route of administration, the optimum target haematocrit, and the long term effects are required in the coming years. An increased understanding of the basic principles of the physiology and pathophysiology of EPO will emerge in this decade.

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