Recombinant human erythropoietin in the treatment of renal anaemia: An update

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Introduction

The isolation of erythropoietin (EPO) from human urine [1], the cloning and expression of its gene in a mammalian cell line [2, 3], and its large-scale synthesis using recombinant DNA technology represent a major advance in the treatment of renal anaemia.

Many excellent reviews and editorials on this subject have already been written [4-16], and for a complete account of the development of the recombinant molecule and its early therapeutic trials the reader is referred to such articles. The aim of the present review intravenous route of administration [17-20], more recent developments. Whereas the early studies were concentrated on haemodialysis patients and the intravenous route of ad- ministration [17-20], more recent reports have examined the use of EPO in the treatment of pre-dialysis patients [21-23] and those on CAPD [24-27] and have also detailed the pharmacokinetics [28, 29] and efficacy [23-27, 30-33] of SC and IP routes of administration. The use of EPO has also highlighted the problem of functional iron deficiency which can have a deleterious effect on the haemopoietic response to such a potent stimulus [8, 9, 18, 34, 35]. In addition to discussing this latter problem, we shall in this article deal with such issues as selection criteria of patients for EPO therapy, possible starting doses and routes of administration, the optimum rate of response and target haemoglobin, the phenomenon of EPO resistance, cardiovascular and haemodynamic effects of EPO, pathogenesis and management of EPO-induced hypertension, other side-effects associated with EPO therapy, and the use of EPO in pre-dialysis patients.

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Which patients should receive EPO therapy?

It remains uncertain exactly how many dialysis patients would benefit from EPO treatment but estimates of between 50 and 75 % have been made [9, 10]. Since the therapy is both expensive and long-term careful consideration has to be given to the selection of appropriate patients.

Several criteria may be used in deciding which dialysis patients should receive EPO. These include the level of haemoglobin, the period of time on dialysis, the presence or absence of symptoms of anaemia, as well as iron status. In general patients who have been on haemodialysis or CAPD for a minimum of 3 months and have a haemoglobin concentration consistently below 8 g/dl should be considered for EPO therapy. Patients who have recently commenced dialysis may experience spontaneous amelioration of their anaemia to more acceptable levels [36-38] thus negating the need for EPO. In contrast a dialysis patient with symptoms of ischaemic heart disease, even if the haemoglobin concentration is above 8 g/dl, should also be considered for EPO therapy.

For patients with chronic renal failure not yet requiring dialysis the situation is even less clear. Relatively few of these patients have a haemoglobin level or less than 8 g/dl unless they are imminently pre-dialysis. Those who do frequently have a multifactorial cause for their anaemia. In addition there remains some concern that the increased blood viscosity resulting from the improved haematocrit [39-41] may adversely affect renal perfusion and this accelerate the decline in renal function [42], although the clinical studies to date do not support this hypothesis [21-23]. Each case of pre-dialysis renal anaemia should be assessed on its own merit, and if the patient is symptomatic then EPO therapy may be appropriate.

In patients with conditions such as SLE, myeloma, sideroblastic anaemia, or who are receiving immunosuppressive therapy, then measuring a basal serum erythropoietin level may be helpful. If this is

inappropriately low for the degree of anaemia, then EPO therapy may be indicated; the response, however, is likely to be impaired. Further experience with the use of EPO in multifactorial anaemia is required before this question can be fully answered since most of the EPO trials to date excluded such cases.

Dosage of EPO and routes of administration

There are now numerous studies published on the treatment of renal anaemia with EPO and a variety of dosage regimens and routes of administration have been employed. By far the greatest experience is with intravenous therapy in haemodialysis patients, and one of the earliest studies showed that there was a dose-dependent rate of response to EPO [18]. However, it has become increasingly apparent that the risk of side-effects such as severe hypertension and thrombotic complications is lessened with a haemoglobin rise not exceeding 1 g/dl/month. As a result the recommended starting dose of EPO has consequently declined in comparison with earlier studies. Most centres now use an initial intravenous dose in the range 100-200 U/kg/week for haemodialysis patients, divided into 2 or 3 doses. A similar IV dosage regimen has been used with good effect in patients not yet on dialysis [21-23].

The intravenous route is clearly impractical for regular use in CAPD patients. Obvious alternatives to be considered include the intraperitoneal and subcutaneous routes. In a single-dose pharmacokinetic study in CAPD patients we compared the IV, IP and SC routes of administration, and found that the bioavailability of SC EPO was seven times greater than that of IP administration, but was still only 22 % [28]. These results were subsequently confirmed by Boelaert et al. [29] Nevertheless, Frenken et al. [24] used the intraperitoneal route for treating 5 CAPD patients, and obtained an effective clinical response with a dose of 300 U/kg/week. We have subsequently shown a similar response with only 120 U/kg/week given subcutaneously in 9 CAPD patients [26].

Bommer et al. [30] have also shown that a 50 % reduction in dose can be achieved at optimal haemoglobin concentration by switching from intravenous to subcutaneous administration. Stevens et al. [25] treated 12 CAPD patients with subcutaneous EPO and obtained a brisk response to a starting dose of 100 or 150 U/kg thrice weekly. The dose was then reduced to 12.5-50 U/kg (median 25 U/kg) thrice weekly to maintain a haemoglobin between 11.0 and 11.5 g/dl. A similarly rapid response was obtained in another study of five transfusion-dependent children on CCPD in whom the haematocrit increased from 22 % to 33 % after only 3 weeks' treatment with EPO given SC at an initial dose of 150 U/kg thrice weekley [33]. A much lower SC dose of 50 U/kg twice weekly was used by Steinhauer et al. [27] to treat eight adult CAPD patients. In predialysis patients, similar responses were achieved with 150 U/kg thrice weekly IV and 100 U/kg thrice weekley subcutaneously [23]. The lowest weekly dose of EPO reported to be effective was also given via the subcutaneous route, in a study by Granolleras et al. [32]. They found that an adequate haemopoietic response was obtained in haemodialysis patients with SC administration of only 14 U/kg daily.

Thus, the subcutaneous route appears to be gaining popularity not only in CAPD patients [25-27] but also in haemodialysis subjects [30-32], and the evidence to date suggests that lower doses of EPO may be used when given by this route. Further dose-finding studies with SC-administered EPO are required, but on the evidence available a starting subcutaneous dose in the range 75-150 U/kg/week in 2 or 3 divided doses seems appropriate. If the patient can be taught to give their own SC injection without stress or discomfort, then the daily dosing regimen [32] may be worth considering.

Target haemoglobin and rate of rise

There is little doubt that it is possible to fully correct the anaemia of chronic renal failure with EPO. In comparing the benefits with the risks, however, partial correction of the anaemia seems the best compromise. A linear increase in the haemoglobin or haematocrit leads to an exponential rise in whole blood viscosity [39-41] which, in turn, is thought to contribute to many of the side-effects of EPO therapy such as hypertension, increased peripheral resistance and thrombotic complications.

The optimum haemoglobin seems to be in the range of 10-12 g/dl. It is at this level that the risk-benefit ratio appears minimised. Nevertheless, this is a very arbitrary guideline, and some flexibility is clearly necessary in treating individual patients. Since the main aim of EPO therapy is to reverse the symptoms of anaemia, differing thresholds at which this occurs may influence the appropriate target haemoglobin.

With regard to the rate of rise of the haemoglobin response, we feel that for most patients an increase of 1 g/dl/month appears sensible. Exceeding this limit may predispose the patient to an increased risk of side-effects, and there is rarely any indication for more rapid correction of anaemia.

Monitoring iron status before and during treatment with EPO

It is important to determine clearly the baseline iron status of any patient being considered for EPO therapy

for two reasons. Firstly, if a patient is frankly iron deficient then one can expect some improvement in the anaemia with iron therapy alone, either oral[43] or intravenous[44]. The potential cost saving following such simple therapy could be substantial. As a result, therapy with EPO should be withheld until it is clear that the patient is not iron deficient, or until a deficiency has been fully treated as judged by a stable haemoglobin concentration. If the serum ferritin is less than 15 µg/l the patient is iron deficient. Higher levels of serum ferritin may be found in iron deficient patients, for example in conditions causing hepatocyte dysfunction where there is significant release of intra-hepatocyte ferritin secondary to tissue damage, or massive resorption of iron from extravasated blood. In these circumstances the serum ferritin may be raised out of proportion to the iron stores. If there is doubt about the patient's true iron status a therapeutic trial of oral or IV iron should be given for a minimum of 4 weeks. If there is any response during this period then EPO therapy should be postponed until a new stable haemoglobin concentration is achieved. At this point the need for EPO should be reassessed. In all cases it is important to remember that infection or active inflammatory processes, including peritonitis in CAPD patients, may prevent a response to iron therapy.

The second reason why it is important to estimate the baseline iron status of a patient commencing EPO therapy is to determine whether there is sufficient readily available iron to meet the anticipated demand. The advent of EPO has resulted in an unprecedented and potent therapeutic stimulus to erythropoiesis, and it has become apparent that abundant quantities of iron are utilised during this process. Thus, patients who are iron replete before starting EPO can rapidly become deficient under the influence of EPO[8, 9, 18, 33-35, 45]. Five of our patients had an initial response to EPO which rapidly tailed off due to an insufficient iron supply to the proliferating marrow[35]. This may occur in the presence of a normal serum ferritin (suggesting adequate iron stores) and stainable iron in the marrow, and the problem appears to be a limitation in the rate of iron supply, i. e. the stores are unable to release iron fast enough to meet the demand.

Clearly, in order to maximise the cost-effectiveness of EPO, it would be useful to predict and counteract this condition of functional iron deficiency in advance. Previous work has shown that a rise of 1 g/dl in the circulating haemoglobin concentration uses 150 mg of storage iron (equivalent to nearly 20 µg/l of serum ferritin)[46, 47]. Thus, for an anticipated haemoglobin rise of 5 g/dl following EPO therapy, the absolute minimum requirements would be 750 mg of storage iron (100 µg/l serum ferritin). Patients with starting serum ferritin levels less than 100 µg/l, therefore, are highly likely to develop functional iron deficiency. Such individuals will require intensive iron supplementation, almost certainly in the form of parenteral iron . Recently, a nomogram was devised for estimating the projected iron deficit based on the initial haemoglobin and serum ferritin, and assuming a target haemoglobin concentration of 11.6 g/dl[34]. The same authors also claimed that oral iron supplementation was unlikely to be able to keep pace with the demand during acute EPO therapy[34].

Patients with initial serum ferritin levels greater than 100 µg/l may also develop functional iron deficiency[18, 33, 34] which is best detected from changes in the percentage transferrin saturation with iron. If the transferrin saturation falls below 20 % then it is likely that the available iron supply to the erythron is inadequate[8, 9, 18, 34, 35]. Bainton & Finch pre- viously demonstrated that functional iron deficiency occurred once the transferrin saturation was reduced to levels below 16 %[48]. This if the transferrin saturation becomes less than 20 % at any stage of EPO treatment, then parenteral iron therapy is indicated.

Figure 1 presents an algorithm for managing patients on EPO therapy, with particular emphasis on the monitoring of iron status and the need for iron supplementation.

EPO resistance

The large multi-centre trials of EPO in the USA and Europe indicate that 95-98 % of patients treated with EPO will respond[45]. Nevertheless, there is undoubtedly a small proportion of patients who have either no response or a grossly inadequate one. Even though some of these patients will respond to a much higher dose of EPO a precipitating cause should first be sought (Table 1). The most common problem is

Table 1. Potential causes of EPO resistance

I. **Decreased RBC production**
   1. Iron deficiency.
   2. B_{12}/folate deficiency.
   3. Aluminium toxicity.
   4. Hyperparathyroidism/marrow fibrosis.
   5. Infection:
      — acute,
      — chronic,
      — occult.
   6. Malignancy:
      — occult?
   7. Poor absorption of EPO (if given SC).
   8. Marrow dysfunction.
   9. Red cell enzyme abnormalities, e.g. pyruvate kinase deficiency.

II. **Decreased RBC survival**
   1. Blood loss:
      — dialysis,
      — other? GI tract? Occult.
   2. Haemolysis.

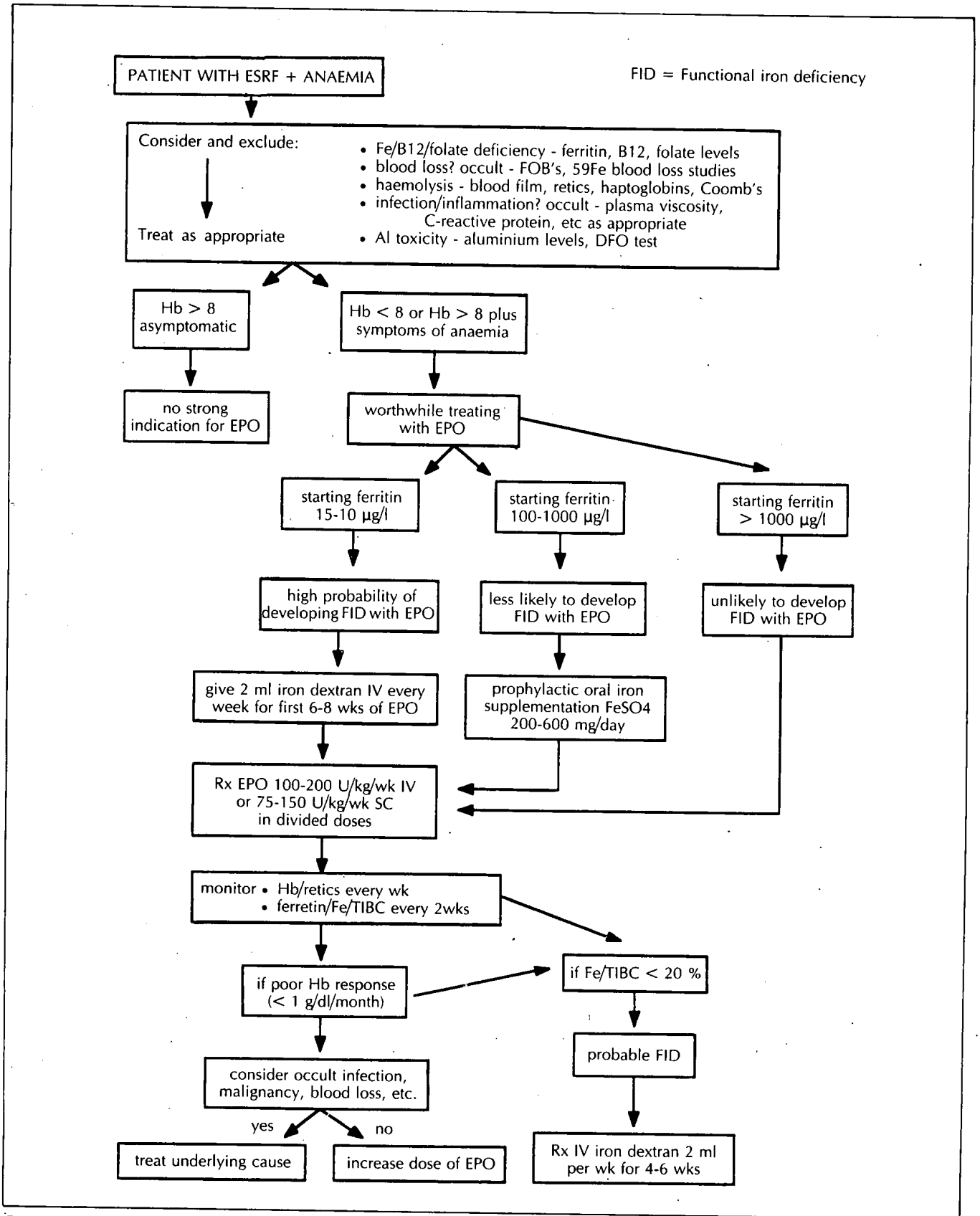

Fig. 1.—Clinical algorithm for treating renal anaemia with EPO (FID = Functional iron deficiency). (Reprinted by permission of the British Medical Journal.)

undoubtedly and inadequate supply of available iron as discussed in the previous section. Other forms of haematinic deficiency, such as B_{12} or folate, are much less common and should have been excluded before commencing EPO therapy. Aluminium toxicity is another cause of EPO resistance, but results from a recent European multi-centre trial showed that this had to be severe before haemopoiesis was inhibited[49]. High PTH levels inhibited erythropoiesis *in vitro*[50], but the clinical relevance of these findings remains controversial[49, 51, 52]. Infection, either acute or chronic, is a potent suppressor of erythropoietic activity and several cases have been reported in whom this has occurred[17]. Thus occult infection should be sought in any patient not responding, or losing a response, to EPO. Similarly, occult malignancy should also be considered. A 66-year-old patient of ours on CAPD was treated with gradually increasing doses of subcutaneous EPO up to 360 U/kg/week and obtained no response at all over a 4 month period. She had had a mastectomy for breast carcinoma 3 and a half years previously but was believed to be disease-free at the time of commencing EPO. She was admitted for investigation of increasing malaise and weight loss, and was found to have liver metastases on ultrasound, biopsy of which confirmed recurrence of her tumour.

All the above causes of EPO resistance represent examples of an inadequate haemopoietic response to EPO. The other possibility is that EPO is effective in initiating increased erythropoietic activity, but that there is also increased red cell loss, either through haemolysis or blood loss (Fig. 2). A clue to this may be an enhanced reticulocyte response to EPO which is not reflected in any change in the haemoglobin.

Thus patients showing a poor response to EPO, or loss of a previous response, require investigation for an underlying cause. Haematinic deficiency, aluminium toxicity, haemolysis and blood loss can be relatively easily excluded, although occult infection or malignancy may prove more difficult. It may be possible to override some of these causes of EPO resistance with a higher dose of EPO, but the importance of excluding them should not be disregarded.

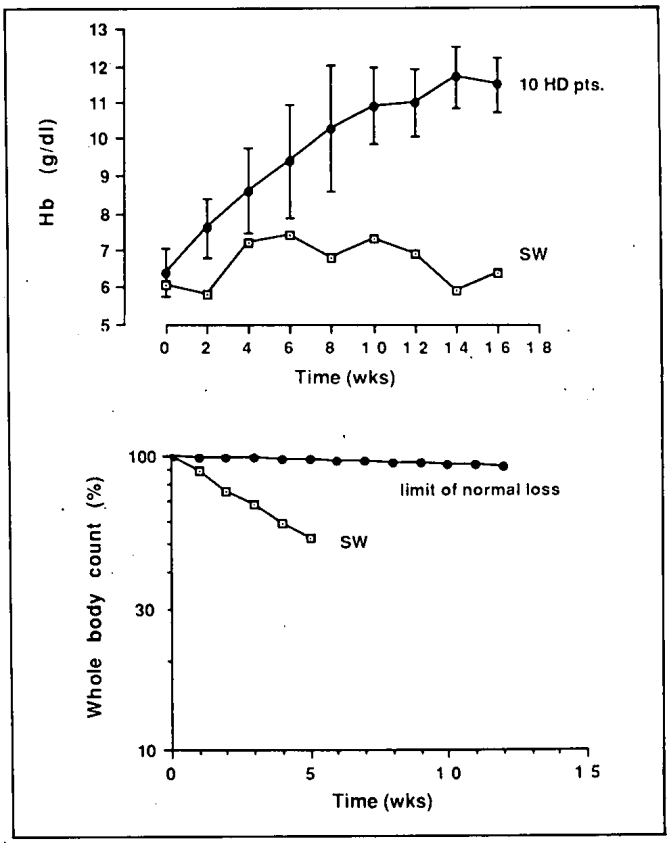

Fig. 2.—Impaired haemoglobin response to EPO in a 34-year-old female patient (SW) who was found to have gastrointestinal bleeding. Lower panel shows quantitative ^{59}Fe blood loss studies which were strikingly positive. Upper G-I endoscopy revealed several gastric erosions as the presumed source of bleeding.

Cardiovascular and haemodynamic effects of EPO

Chronic severe anaemia has profound effects on the cardiovascular system, resulting in an increase in cardiac output, decrease in peripheral resistance due to compensatory vasodilation secondary to tissue hypoxia, and reduced whole blood viscosity[53-57]. In addition, the anaemia of end-stage renal failure is believed to play a major role in the development of left ventricular hypertrophy in patients receiving long-term dialysis[58, 59]. This, in turn, appears to be an important, independent determinant of survival in such patients[60]. Although renal patients with chronic anaemia often exhibit some adaptation and tolerability to their lowered haematocrit, they have nevertheless a grossly impaired exercise capacity, lowered maximal oxygen consumption, and reduced threshold for anaerobic metabolism during physical activity[61-64]. They also have an increased predisposition to coronary artery disease, in part related to abnormal lipoprotein profiles, but almost certainly exacerbated by severe anaemia causing myocardial ischaemia[65-68].

It is likely, therefore, that correction of renal anaemia by EPO would have major effects on cardiovascular function and haemodynamic measurements. It was clearly of some importance to show that such changes would be beneficial to the patients receiving EPO and that the benefits would outweigh any possible deleterious effects.

Studies prior to the advent of EPO therapy showed that acute reversal of uraemic anaemia by red cell transfusion was followed by a reduction in cardiac output and an increase in total peripheral resistance, resulting in an increase in diastolic blood pressure[69].

Although EPO causes a more gradual rise in haematocrit similar effects might be expected. Several studies have assessed the haemodynamic effects in patients treated with EPO. Buckner et al. [70] studied 7 normotensive haemodialysis patients before and after correction of anaemia with EPO, and found a reversal in compensatory vasodilation without complete normalisation of the elevated cardiac output. Akiba et al. [71] performed Swan-Ganz catheterisation in 12 haemodialysis patients before and after 12 weeks of EPO, and observed a significant fall in cardiac index along with increases in blood viscosity and systemic vascular resistance. Similar results have been obtained by others [72, 73], although one group inexplicably found an increase in cardiac output and decrease in peripheral resistance after EPO [74].

A number of studies have confirmed the expected increases in working capacity, maximal oxygen consumption, and anaerobic threshold after EPO therapy [75-80], consistent with the subjective improvement in the patients' physical well-being and exercise capacity. Mayer et al. [78] found acute improvements in both aerobic and anaerobic capacity assessed using bicycle spiro-ergometry, after the target haemoglobin of 10 g/dl had been attained. A similar acute increase in maximal oxygen uptake (VO_2 max) was observed by Tsutsui et al. [79] and ourselves [80] using multi-stage treadmill testing, although only in the latter study was this maintained on subsequent re-assessments. In the same group of patients, we found that 7 out of 10 patients had ECG evidence of myocardial ischaemia during exercise before EPO therapy; this was reversed in all but one after 4 months of EPO, and by 12 months none of the patients had any evidence of an ischaemic ECG during exercise [80]. Acute reversal of myocardial ischaemia after 3 months of EPO was also observed by Wizemann in 8 haemodialysis patients [81].

Since it has been suggested that chronic anaemia is a major determinant of left ventricular hypertrophy in long-term haemodialysis patients [58, 59], there are strong theoretical reasons why reversal of renal anaemia by EPO might result in a decrease in LVH. We noted a significant progressive reduction in LV mass, measured using echocardiography, in 10 haemodialysis patients monitored over the first 12 months of EPO therapy [80]. A similar regression of LVH was recently reported following renal transplantation [82]. Other echocardiograhic parameters have also been shown to improve after EPO therapy [83].

Pathogenesis and management of hypertension related to EPO therapy

Hypertension has been the most frequently reported side-effect associated with EPO therapy [8-10, 14-20, 39, 84]. Virtually all clinical studies published to date have documented some exacerbation of hypertension in patients trated with EPO. Casati et al. [20] found that all eight previously hypertensive patients in their study required treatment for elevated blood pressure whereas none of their six normotensive patients demonstrated any change in BP during EPO therapy. In contrast, results of a multi-centre clinical trial of EPO in 309 evaluable patients showed that although 72 % of the patients had existing hypertension at baseline, they proved to be at no greater risk of increased blood pressure than those who were normotensive to begin with. Only 35 % of the patients in the study developed sustained increases in diastolic BP of 10 mmHg or more [84].

Despite a strong association between EPO therapy and hypertension, there is no evidence to suggest that EPO itself has a haemodynamic effect. Thus, the increase in blood pressure is thought to be mediated via a number of pathophysiological changes occurring secondary to the increase in haematocrit. Three such mechanisms have been suggested as being contributory: increased peripheral vascular resistance, increased blood viscosity, and inadequate reversal of the elevated cardiac output of anaemia.

A significant increase in peripheral resistance was found by Neff et al. [69] after haematocrit levels were increased from 20 % to 40 % by red cell transfusion over a 3 week period. It was concluded that hypoxic peripheral vasodilation, which occurs as a compensatory response in chronic anaemia, is reversed as the anaemia is corrected, resulting in relative vasoconstriction which increases the peripheral resistance. Similar effects on peripheral vascular resistance have been found during correction of renal anaemia by EPO [70-73].

Another mechanism which may also contribute to the increase in peripheral resistance and blood pressure is the rise in blood viscosity associated with the increase in haematocrit [39-41]. As the haematocrit rises in a linear manner, there is an exponential rise in whole blood viscosity. In addition there is a direct relationship between blood viscosity and vascular resistance [42, 85]. Finally, although there is usually a normalisation of the elevated cardiac output as the anaemia is corrected, occasionally this does not occur [70], and a sustained high cardiac output in the presence of a reversal of the compensatory vasodilation of anaemia would result in an increase in blood pressure.

In view of the important role of vasoconstriction in the aetiology of hypertension associated with EPO therapy, it might seem logical to use anti-hypertensive agents that reduce peripheral vascular resistance, e.g. the α-adrenergic inhibitors, calcium channel blockers, and ACE-inhibitors. In the majority of instances, blood pressure can be easily controlled by

the use of these agents, and it is very rare to have to discontinue EPO therapy for severe uncontrollable hypertension [84]. However, EPO should be stopped immediately if hypertensive encephalopathy ensues. More importantly, however, the aim should be to increase the haemoglobin gradually by the use of an appropriate dosage regimen of EPO with a view to reducing the chances of hypertension.

Other side-effects and complications of EPO

A further major complication of EPO therapy is thrombosis of the arteriovenous fistula which occurred in 9.3 % of haemodialysis patients entered into a European multicentre study [86]. Many other published reports have documented small but significant numbers of patients experiencing this problem [17, 19, 20], often early in the course of EPO treatment. For a long time there was doubt over whether there was a true increase in the incidence of fistula thrombosis with EPO but a recent placebo-controlled multicentre trial suggested that there was a significantly increased risk of this complication developing in patients receiving EPO [87]. As with hypertension, the rise in haematocrit and associated increase in blood viscosity are thought to be contributory, although the absolute levels of blood viscosity attained at the target haemoglobin are still considerably lower than those from normal subjects. Other factors which may contribute to an increased predisposition to thrombosis include alterations in platelet function [88-91] and a reduction in protein C and protein S levels in patients treated with EPO [92].

Less serious side-effects noted during EPO therapy include transient myalgia and flu-like symptoms occurring shortly after administration of the first few doses of EPO [17, 20, 45, 86], conjunctival injection [45], and headache. In addition there have been a few reports of nausea, vomiting, shortness of breath, diarrhoea, and abdominal or loin pain [17, 18, 93], but the non-specific nature of these symptoms makes accurate assessment very difficult, and their relationship to EPO remains unclear at the present time. Genuine intolerance to EPO sufficient to warrant stopping the hormone is rare. To date there have been no reports of antibody formation [94].

In some haemodialysis patients higher pre-dialysis potassium and phosphate levels accompany the rising haematocrit [18, 20]. This may be due to increased dietary intake resulting from the general improvement in well-being. However, it is possible that dialyser potassium clearance is lower with higher haemoglobin concentrations [95]. Thus, dietary guidelines should be reinforced for all subjects starting EPO treatment. A mild increase in platelet count has been observed in some studies with EPO [19].

EPO in pre-dialysis patients

Recent investigations have confirmed the efficacy of EPO in reversing the anaemia of end-stage renal failure in patients not yet requiring renal replacement therapy [21-23]. Lim et al. [21] treated 14 anaemic pre-dialysis patients with intravenous EPO in double-blind placebo-controlled trial and observed an increase in the mean haemoglobin from 9.1 ± 0.2 (SE) to 12.3 ± 0.4 g/dl over a 2 month period. In a further study by Eschbach et al. [23] all 17 pre-dialysis patients with anaemia responded to EPO with a median haematocrit rise from 0.27 to 0.37. Similar results were obtained by Stone et al. [22]

The major concern in this group of patients was that the resultant increase in blood viscosity and possible exacerbation of hypertension might cause a more rapid decline in renal function [42], thereby forcing patients to start dialysis sooner than they might otherwise have done. Indeed, studies in 5/6 nephrectomised rats suggested that renal function deteriorated more rapidly in EPO-treated rats than in non-EPO treated rats [96, 97]. This has not been borne out by the human studies that have been conducted to date. Lim et al. [21] found no change in renal function as judged by blood urea nitrogen, serum creatinine, and creatinine clearance, although the follow-up was only for 2 months. Eschbach et al. [23] likewise observed no change in the rate of deterioration in renal function measured by the reciprocal of the serum creatinine over 6 months in 17 pre-dialysis patients treated with EPO. Longer term studies will be required, however, before the safety of EPO in pre-dialysis patients can be more confidently established.

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