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Darbepoetin alfa: A new therapeutic agent for renal anemia

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Darbepoetin alfa: A new therapeutic agent for renal anemia. Darbepoetin alfa is a super-sialylated analog of human erythropoietin that has a longer circulating half-life in vivo compared to both native and recombinant hormone. It has the same mechanism of action as erythropoietin, stimulating the same surface membrane receptor and triggering the same intracellular chain of events. An extra two N-linked carbohydrate chains, however, gives darbepoetin alfa greater metabolic stability in vivo, and its terminal half-life after intravenous administration is approximately three times longer than for intravenous erythropoietin. This in turn allows injections of the drug to be given less frequently, and studies have shown that once-weekly and once-every-other-week dosing can maintain the hemoglobin concentration in patients with renal anemia. The recommended starting dose for darbepoetin alfa is 0.45 μg/kg once weekly for both IV and SC administration, with subsequent titration based on the hemoglobin concentration. The adverse event profile is very similar to that seen with rHuEPO, and no antibodies have been detected in several thousand patients exposed to the drug, some of whom have been treated for up to five years now. Following a clinical research program that began in November 1996, darbepoetin alfa was finally approved by the European Commission in June 2001, and by the FDA in September 2001.

The advent of recombinant human erythropoietin (rHuEPO) as a therapeutic agent for the treatment of renal anemia in the late 1980s transformed the management of this condition, which often previously relied on repeated blood transfusions. Treatment with rHuEPO, which represents supplementation of a hormone-deficient state, has proved highly effective in over a million patients treated worldwide, and this has been associated with significant improvements in general well-being, quality-of-life, exercise tolerance, and cardiac function. Initial studies conducted in Seattle [1] and London/Oxford [2] employed a thrice-weekly intravenous-dosing regimen that coincided with the visits of the hemodialysis patients to the Renal Unit. The relatively short elimination half-life after IV administration of around four to eight hours suggested a rationale for this dosing regimen [3, 4], and some units (particularly in the USA) continue to use IV rHuEPO three times a week. The use of the subcutaneous route for rHuEPO administration was first reported in 1988 [5], and most of the evidence suggests that 20 to 30% lower weekly dosages might be required compared with intravenous administration [6]. The subcutaneous route is also the only practical one for administering rHuEPO to peritoneal dialysis and pre-dialysis patients. Although the elimination half-life after subcutaneous administration is longer than with IV dosing due to the continuing absorption from the site of injection, most units have adopted twice- or thrice-weekly dosing regimens.

As with other glycoprotein hormones, it was recognized that the sialic acid residues on erythropoietin were critical to its metabolic stability in vivo [7]. Once the hormone is desialylated, erythropoietin is cleared very rapidly from the circulation, probably via galactose receptors in the liver [8]. It also was recognized that the greater amount of sialic acid residues on the molecule, the longer the serum half-life, and the greater the degree of in vivo biological activity [7]. This led to the hypothesis that increasing the carbohydrate content of naturally occurring erythropoietin would produce a molecule with an even longer circulating half-life in vivo, with the potential for enhanced biological activity. This was the rationale for creating a scientific research program that culminated in the design and large-scale synthesis of novel erythropoiesis-stimulating protein, now called darbepoetin alfa.

DEVELOPMENT OF DARBEPOETIN ALFA

Human erythropoietin is a 30,400-dalton glycosylated protein containing a 165 amino acid residue backbone [9]. Attached to this, as a post-translational event, are three N-linked and one O-linked carbohydrate chains. Each of the N-linked chains can contain a maximum of four sialic acid residues, and the O-linked chain can contain up to two sialic acid residues. The erythropoietin molecule, therefore, may contain anywhere from 4 to 14 sialic acid residues [10].

These different isoforms of human erythropoietin may be separated on a gel using iso-electric focusing, based on the highly negatively charged sialic acid residues.

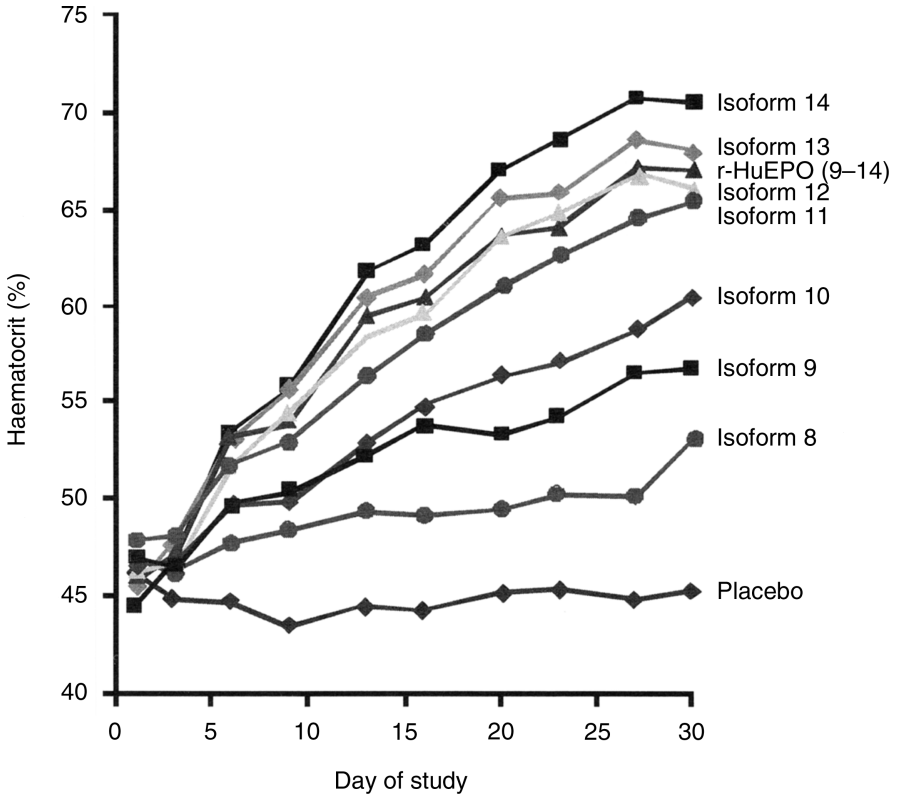

Fig. 1. Hematocrit response following thrice-weekly intraperitoneal injection of various isolated recombinant human erythropoietin (rHuEPO) isoforms in mice. (Data are from Egrie et al, *Glycoconj J* 10:263, 1993 [7]; used with permission.)

Animal experiments in the early 1990s suggested that these different isoforms of erythropoietin had different biological potency in vivo [7]. Thus, when injected into mice intraperitoneally three times a week, isoform 14 showed a much greater hematocrit response than did isoform 8 (Fig. 1) [7]. This is despite the fact that isoform 14 has a reduced receptor binding affinity for the erythropoietin receptor compared to the smaller erythropoietin isoforms.

The explanation for this effect lies in the metabolic clearance of the molecule in vivo. Another animal experiment conducted by Egrie et al showed that isoform 14 was cleared very much more slowly from the circulation when injected intravenously into rats, compared to isoform 6 (3.8 mL/kg $\times$ h compared to 26.8 mL/kg $\times$ h; Fig. 2) [7]. These interesting findings led to the hypothesis that if additional sialic acid residues could be attached to human erythropoietin, then a molecule with an even longer circulating half-life may be created.

The strategy for achieving this involved using site-directed mutagenesis to introduce extra N-linked glycosylation consensus sequences into the molecule [11]. These consensus sequences consisted of three amino acids: (*1*) asparagine, (*2*) any amino acid, and (*3*) a threonine or serine residue. The plan then was to identify the individual variants of rHuEPO that had the desired properties, namely N-linked glycosylation along with similar molecular folding and stability. These rHuEPO variants could then be tested for in vitro and in vivo activity.

Before undertaking this endeavor, however, it was important to determine the appropriate site for the introduction of the extra N-linked chains. In particular, it was essential that there was no loss of biological activity, and so a series of laborious experiments were conducted to investigate the effect of changing one amino acid at a time. A map of the erythropoietin molecule was created, and a three dimensional model of this suggested that there were two main sites essential for biological activity of the molecule. These corresponded to the two binding domains with the erythropoietin receptor [11].

Many new molecules were created, one of them being NM279, which contained four N-linked carbohydrate chains. This showed greater in vivo activity using a mouse ex-hypoxic polycythemic mouse bioassay. Continuing experiments, however, led to the development of NM321 (darbepoetin alfa), which contained five N-linked carbohydrate chains, the additional two chains attaching at asparagine residues 30 and 88 (Fig. 3). To achieve this end, five amino acid substitutions were made to the native molecule at the following positions: Ala30Asn, His32Thr, Pro87Val, Trp88Asn and Pro90Thr [11]. The introduction of the two extra N-linked oligosaccharide chains increased the potential maximum number of sialic acid residues from 14 up to 22. The extra carbohydrate

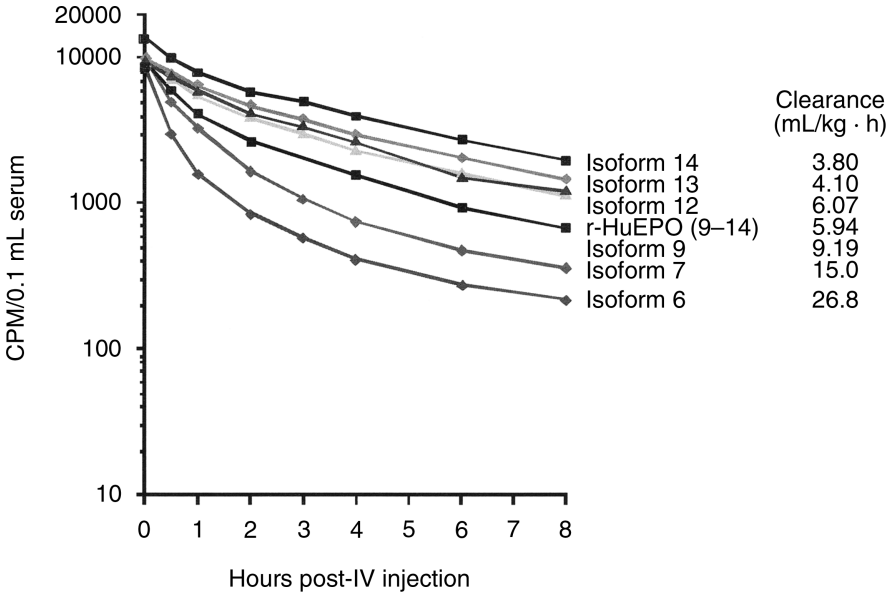

Fig. 2. Intravenous pharmacokinetics of isolated rHuEPO isoforms in rats. (Data are from Egrie et al, *Glycoconj J* 10:263, 1993 [7]; used with permission.)

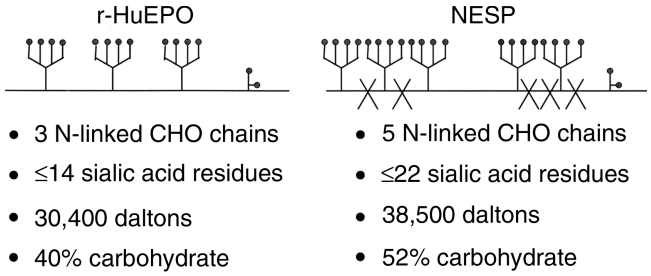

Fig. 3. Comparison of the structure of darbepoetin alfa and rHuEPO. The "X"s in darbepoetin alfa represent the five amino acid exchange sites that were required to allow the attachment of two extra N-linked carbohydrate chains.

on darbepoetin alfa also increased the molecular weight of the molecule from 30,400 (for native erythropoietin) up to 38,000. The carbohydrate content of darbepoetin alfa is approximately 52%, compared to around 40% for erythropoietin. In vitro, the affinity of darbepoetin alfa for the erythropoietin receptor is less than for the natural ligand, but this is more than compensated for by the increased potency in vivo [11].

PRE-CLINICAL STUDIES

One of the earliest experiments compared the pharmacokinetics of IV darbepoetin alfa with IV rHuEPO in rats (abstract; Egrie et al, *Blood* 90:56a, 1997). The mean terminal half-life in vivo was 6.9 hours for darbepoetin alfa compared with 2.5 hours for rHuEPO, with a correspondingly reduced clearance rate (4.8 vs. 17.7 mL/kg × h). The same experiment was conducted in dogs, and again an approximately threefold longer half-life was obtained for darbepoetin alfa compared to rHuEPO (25.0 vs. 7.2 h). The mean clearance of darbepoetin alfa in dogs was 2.4 mL/kg × h compared to 8.4 mL/kg × h for rHuEPO (abstract; *ibid.*).

In another series of experiments, Egrie et al examined the changes in hematocrit in mice associated with once-weekly intravenous dosing of darbepoetin alfa (7.5 and 15 μg/kg/week) and compared the results with the same weekly dose of rHuEPO administered thrice weekly (abstract; *ibid.*). Using a *cis*-platinum-induced animal model of anemia in rats, Akahori et al found that weekly IV administration of darbepoetin alfa (1 μg/kg/day) for six weeks resulted in complete correction of the hematocrit by the second week of treatment (abstract; *Exp Hematol* 26:766, 1998). Again, single weekly doses of darbepoetin alfa were as effective as rHuEPO given three times weekly. The efficacy of darbepoetin alfa in increasing the hemoglobin concentration also was confirmed in another anemic rat model induced by immunization with a peptidoglycan-polysaccharide polymer (abstract; Cooke et al, *Blood* 96:8a, 2000). The dose used in their study was 30 μg/kg every two weeks, and again complete correction of the anemia was achieved within 28 days of initiating treatment.

PHARMACOKINETIC STUDIES IN HUMANS

The first single-dose pharmacokinetic study of darbepoetin alfa in humans was initiated in November 1996 [12]. This involved 11 stable continuous ambulatory peritoneal dialysis (CAPD) patients who were given a single intravenous injection of epoetin alfa (100 U/kg) and the equivalent peptide mass of darbepoetin alfa in a double-blind randomized manner using a 2 × 2 crossover design. Following a suitable washout period, the absorption of

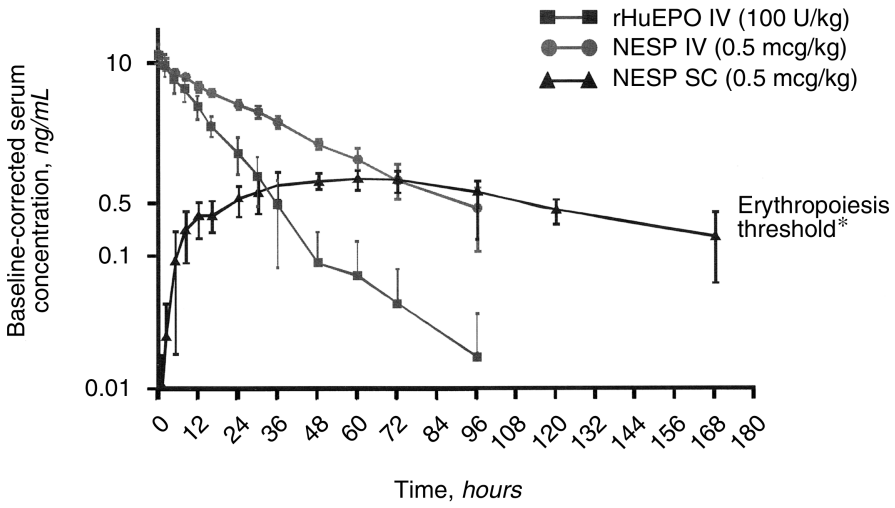

Fig. 4. Pharmacokinetics of darbepoetin alfa injected intravenously and subcutaneously, compared with rHuEPO injected intravenously at an equivalent peptide mass in 11 peritoneal dialysis patients. Data are from [10]; erythropoiesis threshold data are based on the range from the study of Besarab et al [13].

the same dose of darbepoetin alfa injected subcutaneously was assessed, and compared with the IV pharmacokinetics of the drug. As with the animal studies, the mean terminal half-life of IV darbepoetin alfa was threefold longer than for IV rHuEPO (25.3 vs. 8.5 hours), a difference of 16.8 hours (95% confidence interval, 9.4 to 24.2 hours; $P = 0.0008$; Fig. 4). The area under the serum concentration-time curve was significantly greater for darbepoetin alfa (291.0 ± 7.6 vs. 131.9 ± 8.3 ng · h/mL; $P < 0.0005$), and clearance was significantly lower (1.6 ± 0.3 vs. 4.0 ± 0.3 mL/h/kg; $P < 0.0005$). The volume of distribution was similar for darbepoetin alfa and rHuEPO (52.4 ± 2.0 vs. 48.7 ± 2.1 mL/kg) and is approximately equivalent to plasma volume [12].

In phase 2 of this study, six patients received 0.5 μg/kg of darbepoetin alfa subcutaneously. Serum levels increased fairly slowly, reaching a peak at around 54 hours after injection, and the mean maximum serum concentration was approximately 10% of that obtained after the same intravenous dose (Fig. 4). The mean half-life of darbepoetin alfa after subcutaneous administration was 48.8 hours (range 33.5 to 68.0 hours), which was twice as long as that for the intravenous route. At 168 hours (7 days) after the SC injection, the mean levels of darbepoetin alfa were still significantly above baseline. The mean bioavailability of darbepoetin alfa was 36.9%, which was similar to that previously shown for rHuEPO [12]. Thus, a single dose of subcutaneous darbepoetin alfa equivalent to 100 U/kg of rHuEPO was able to maintain serum concentrations above 0.1 ng/mL for up to seven days, a level considered sufficient to stimulate erythropoiesis in anemic patients [13].

The concern with a longer-acting erythropoietic agent might be that levels of the drug would accumulate with repeated dosing. Evidence from multiple dose pharmacokinetic studies, however, has shown minimal accumulation of darbepoetin alfa when investigated in over 700 patients with both intravenous and subcutaneous administration for up to one year [14].

CLINICAL STUDIES OF EFFICACY

To date, several clinical trials have reported the effects of darbepoetin alfa in treating patients with renal anemia, both those on, and those not yet requiring, dialysis (abstracts; Macdougall et al, *J Am Soc Nephrol* 9:258A–259A, 1998; Coyne et al, *J Am Soc Nephrol* 11:1380, 2000; Vanrenterghem et al, *J Am Soc Nephrol* 10:270A, 1999; Nissenson et al, *J Am Soc Nephrol* 11:252A, 2000; Graf et al, *J Am Soc Nephrol* 11:250A, 2000) [15]. Two studies were set up concurrently, with almost identical protocols (abstract Macdougall et al, *ibid.*) [15]. These were randomized dose-escalation studies examining the efficacy of intravenous darbepoetin alfa in hemodialysis patients and subcutaneous darbepoetin alfa in peritoneal dialysis patients. Both protocols involved randomization of patients to either once-weekly or thrice-weekly darbepoetin alfa administration, using four sequential dosing regimens (0.075, 0.225, 0.45, and 0.75 μg/kg/week). Seventy-eight patients were recruited to the hemodialysis study, and 58 patients were recruited to the peritoneal dialysis study. There was a dose-dependent increase in hemoglobin in both studies with no apparent difference between once and three times weekly dosing with darbepoetin alfa (Figs. 5 and 6). Doses of 0.45 and 0.75 μg/kg/week provided optimal responses in 60 to 70% of patients (defined as a hemoglobin increase of 1 to 3 g/dL over the first 4 weeks). These results indicated a dose-related erythropoietic response to darbepoetin alfa over the first four weeks of treatment, and they also suggested an appropriate starting dose of the drug in the region of 0.45 to 0.75 μg/kg/week, administered either intravenously or subcutaneously (abstract; Macdougall et al, *ibid.*).

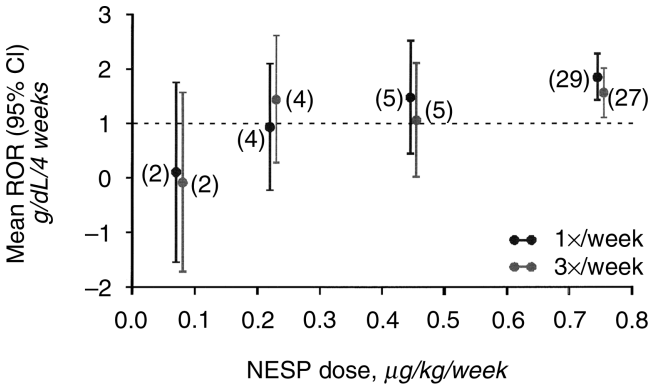

Fig. 5. Dose-response relationship for darbepoetin alfa injected intravenously to hemodialysis patients. ROR is the rate of rise of hemoglobin in g/dL over the first 4 weeks of treatment.

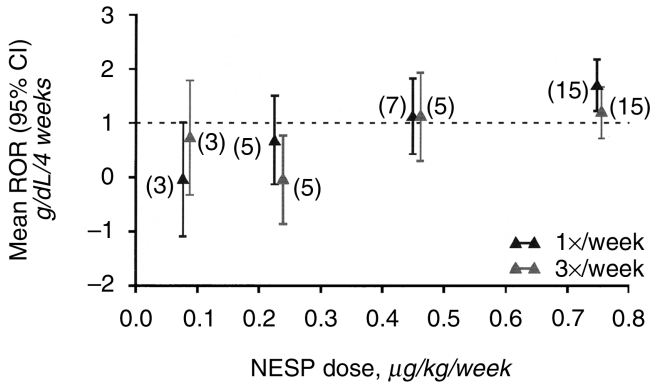

Fig. 6. Dose-response relationship for darbepoetin alfa injected subcutaneously to peritoneal dialysis patients. ROR is the rate of rise of hemoglobin in g/dL over the first 4 weeks of treatment.

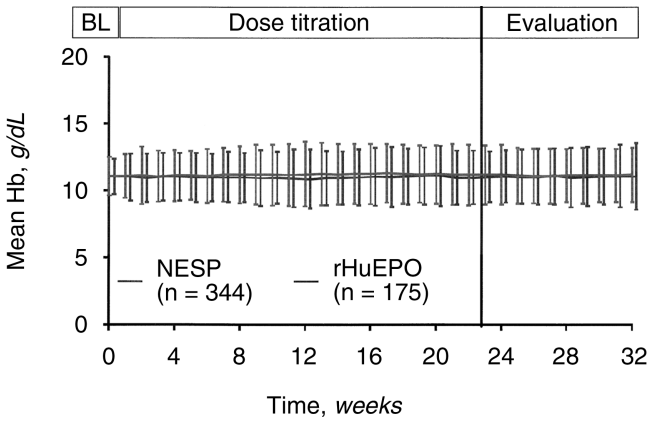

Fig. 7. Comparison of darbepoetin alfa and rHuEPO in maintaining hemoglobin levels in a randomized controlled conversion study.

Two further studies examining the correction of anemia with darbepoetin alfa were then undertaken (abstract; Coyne et al, *ibid.*) [15]. The first of these was in patients with chronic renal insufficiency not yet requiring dialysis [15], and the second of these was in dialysis patients (abstract, Coyne et al, *ibid.*). The starting dose of darbepoetin alfa in these two studies was 0.45 μg/kg/week, and the primary end-point was the proportion of patients achieving a satisfactory hemoglobin response. One hundred sixty-six patients were recruited to the chronic renal insufficiency (CRI) study [15], of whom 129 received darbepoetin alfa (0.45 μg/kg once weekly SC), and the remaining 37 received rHuEPO (50 U/kg twice weekly SC). The proportion of patients achieving a satisfactory hemoglobin response was similar in the two treatment groups (93% darbepoetin alfa, 92% rHuEPO).

In the dialysis study, 122 patients were randomized, of whom 91 received darbepoetin alfa (0.45 μg/kg once weekly IV or SC) and 31 patients received rHuEPO (50 U/kg three times weekly IV or SC). The mean increase in hemoglobin over the initial four weeks of treatment was 1.10 g/dL in patients receiving darbepoetin alfa, and 1.33 g/dL in patients receiving rHuEPO. By 20 weeks, the hemoglobin concentrations in both treatment groups were within the targeted range of 11 to 13 g/dL (abstract; Coyne et al, *ibid.*).

Two studies have examined the effect of switching patients from rHuEPO to darbepoetin alfa (abstracts; Vanrenterghem et al and Nissenson et al, *ibid.*). Thirty-one centers participated in the European/Australian study that treated both hemodialysis and peritoneal dialysis patients (abstract; Vanrenterghem et al; *ibid.*). Patients on twice- or thrice-weekly rHuEPO were converted to once-weekly darbepoetin alfa, and patients on once-weekly rHuEPO were converted to darbepoetin alfa once every other week. In total, 522 patients were randomized (347 on darbepoetin alfa and 175 on rHuEPO). The mean hemoglobin remained stable from baseline to the evaluation period for both treatment groups, and there was no significant difference between the groups (Fig. 7). At the end of the evaluation period, 97% of patients receiving once-weekly darbepoetin alfa and 95% of patients receiving once-every-other-week darbepoetin alfa were successfully treated at the reduced dosing frequencies (abstract; Vanrenterghem et al; *ibid.*).

The North American study was conducted in a double-blind manner, and a total of 507 hemodialysis patients were randomized to receive either IV darbepoetin alfa once weekly plus placebo twice weekly, or to continue on intravenous rHuEPO thrice weekly (abstract; Nissenson et al, *ibid.*). The mean hemoglobin remained stable in both treatment groups from baseline to the evaluation period, and again there was no statistically significant or clinically relevant difference between the two groups.

The conclusion from both of these studies was that darbepoetin alfa was as effective as rHuEPO in maintaining hemoglobin levels in dialysis patients, but with less frequent dosing. A further open-label study of 703

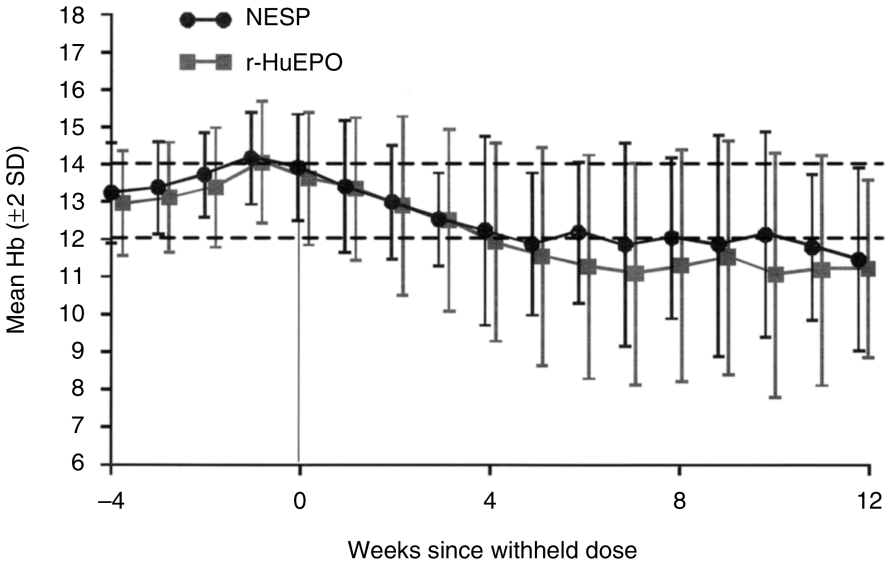

Fig. 8. Decline in hemoglobin concentrations after withholding darbepoetin alfa or rHuEPO in patients whose hemoglobin was increasing too excessively.

dialysis patients conducted in Europe and Australia also confirmed the ability of darbepoetin alfa to maintain hemoglobin levels over a treatment period of one year (abstract; Graf et al, *ibid.*).

ADVERSE EVENT PROFILE

From the clinical trials that have been reported, it has become apparent that darbepoetin alfa possesses an adverse event profile very similar to that of rHuEPO. The data to support this come from controlled studies involving 1578 patients receiving darbepoetin alfa and 591 patients receiving rHuEPO for up to 24 months [16]. The overall proportion of patients who discontinued treatment due to adverse events was 2% for darbepoetin alfa and 4% for rHuEPO. There were no significant differences in the incidence of hypertension (23 vs. 26%), cerebrovascular disorders (<1 vs. 1%), convulsions (1 vs. 2%), myocardial infarction (2 vs. 2%), vascular access thrombosis (8 vs. 14%), and transient ischemic attack (<1 vs. <1%) for darbepoetin alfa compared with rHuEPO. Injection site pain was reported in a number of patients receiving darbepoetin alfa subcutaneously, but this was generally mild and transient in nature. The percentage of patients with hemoglobin levels defined as unstable were similar for darbepoetin alfa (35%) and rHuEPO (38%) [16]. In patients whose hemoglobin concentration increased to above 14 g/dL, treatment with darbepoetin alfa or rHuEPO was stopped. The subsequent fall in hemoglobin concentration was similar for 32 patients receiving darbepoetin alfa and 15 patients receiving rHuEPO in the clinical trials where this problem occurred (Fig. 8) [14].

ANTIBODIES AGAINST DARBEPOETIN ALFA

As stated previously, the amino acid sequence of darbepoetin alfa differs from that of native erythropoietin at five positions, and it is theoretically possible that these alterations could make darbepoetin alfa more immunogenic. To this end, all patients participating in clinical trials of darbepoetin alfa had antibodies tested at three monthly intervals. To date, there have been no antibodies detected against darbepoetin alfa in any patient receiving treatment for anything up to nearly five years. There are also two theoretical reasons why antibody formation might be less of a concern than it might at first seem. First, the tertiary structure of darbepoetin alfa protects the amino acid exchange sites by the carbohydrate side-chains. The latter act as a shield over the potentially immunogenic amino acid exchange sites.

Secondly, the five amino acid substitutions have been made at a site distinct from the two receptor binding sites. This means that any antibodies developing against darbepoetin alfa would potentially be non-neutralizing, and thereby have no effect on the biological activity of the molecule. Thus, darbepoetin alfa could still function as an erythropoietic agent.

CONCLUSIONS

The development of darbepoetin alfa arose out of a hypothesis that increasing the sialic acid content of erythropoietin would generate a molecule that was biologically more active in vivo. The extra sialylation of the molecule was achieved by introducing N-linked glycosylation consensus sequences using site-directed mutagenesis. Darbepoetin alfa is indeed metabolically more stable in vivo, with a threefold longer half-life than native or

recombinant erythropoietin. The clinical implication of this is that patients may obtain the same benefits of anemia correction but with less frequent injections compared with rHuEPO therapy. The studies reported to date have confirmed that patients may be maintained on only once-weekly or even once-every-other-week dosing, and this seems to be true for both IV and SC administration. However, as yet there are no head-to-head studies comparing once-weekly darbepoetin alfa with rHuEPO, and this would be of enormous interest to the nephrological community. In the meantime, clinical experience with darbepoetin alfa in the treatment of renal anemia is increasing, as it is in the oncology setting.

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