Clinical implications of converting stable haemodialysis patients from subcutaneous to intravenous administration of darbepoetin alfa

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Abstract
Background. The erythropoiesis-stimulating protein darbepoetin alfa (Aranesp®) can be given intravenously (IV) or subcutaneously (SC). Despite a SC bioavailability of only 37%, darbepoetin alfa IV or SC dose requirements were comparable in previous studies designed to evaluate other aspects of anaemia treatment. The present study was designed to compare IV vs SC dose requirements.

Methods. A single-centre open-label, prospective and randomized crossover study was undertaken in 71 stable haemodialysis patients. After a run-in period randomized to a 20 week study treatment with either SC or IV darbepoetin alfa, the patients were crossed over to the other treatment modality for another 20 week study period. The unit dose of weekly darbepoetin alfa was adjusted to maintain each patient’s haemoglobin within a target range of −0.8 to +0.8 mmol/l of the individual baseline haemoglobin and between 6.8 and 8.5 mmol/l throughout the study period. The primary endpoint was the mean dose of darbepoetin alfa necessary to maintain the haemoglobin level in the defined range.

Results. Data from 58 patients were available for analysis. Haemoglobin concentrations were maintained effectively in subjects, regardless of whether they received darbepoetin alfa IV or SC. The overall mean difference in haemoglobin levels during SC or IV was 0.052 mmol/l (95% confidence interval: −0.132 to 0.236 mmol/l). The difference had no statistical or clinical significance. The population mean darbepoetin alfa dose during IV treatment was 32.1 mg/week, compared with a mean value for SC treatment of 34.1 mg/week. A paired two-tailed ratio t-test showed that P = 0.036, indicating a 95% probability of a mean dose reduction between 1.2% and 28% by IV treatment instead of SC.

Conclusions. Renal anaemia of stable haemodialysis patients can be treated with darbepoetin alfa more effectively by the IV as compared with the SC route.

Keywords: darbepoetin alfa; haemodialysis; haemoglobin; renal anaemia; route of administration

Introduction
Darbepoetin alfa (Aranesp®) contains five N-linked carbohydrate chains as compared with three in recombinant human erythropoietin (rHuEpo) and darbepoetin alfa contains up to 22 sialic acid residues as compared with rHuEpo’s up to 14 sialic acid residues. These changes in the darbepoetin alfa molecule were introduced to increase the serum residence time and in vivo bioactivity of the protein [1]. Data on the single-pool pharmacokinetics of intravenous (IV) and subcutaneous (SC) darbepoetin alfa are available for adults and children with renal failure. The SC bioavailability was ~37%, while the elimination half-life was 21 and 49 h for IV and SC darbepoetin alfa, respectively [2].

Treatment of renal anaemia with darbepoetin alfa administered by IV or SC routes proved effective in maintaining stable haemoglobin concentrations [3]. However, in contrast to rHuEpo where IV dosing requirements are ~30% higher than for SC dosing [4,5], no obvious and significant differences in darbepoetin alfa dosing requirements were observed for these routes of administration in a number of controlled studies [3–5], despite a lower bioavailability of SC darbepoetin alfa. Nevertheless, these studies were not designed specifically to test for a possible difference in dose efficacy between SC or IV.

Thus, the objective of the current study was to test the null hypothesis of no difference in darbepoetin alfa dosing requirement, IV vs SC.
Subjects and methods

This single-centre open-label, prospective and randomized crossover study in 71 haemodialysis patients evaluated the hypothesis that there is no difference in the dose necessary to obtain a stable level of haemoglobin using IV or SC darbepoetin alfa (Aranesp®; Amgen, Thousand Oaks, CA, USA), respectively.

Patients were included if they fulfilled the following criteria: clinically stable patients (≥18 years) receiving haemodialysis ≥3 months, with a stable dose of rHuEpo or darbepoetin alfa for ≥8 weeks (defined as <20% change in dose), stable haemoglobin concentration in the range 6.8–8.5 mmol/l and a sufficient iron supply [serum ferritin ≥200 μg/l or transferrin saturation (TSAT) ≥20%].

Patients were excluded if they had received haemodiafiltration, home haemodialysis or undergone major surgery during the last 3 months (excluding vascular access surgery), had ongoing infection, evidence of uncontrolled hyperparathyroidism [parathyroid hormone (PTH) >70 pmol/l], clinical signs of current malignant disease or systemic haematological disease, received red blood cell transfusion <8 weeks before screening, active bleeding or lack of compliance. Also, pregnant or lactating females were excluded.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local ethics committee. All patients gave written informed consent before participation.

All patients who met the inclusion criteria received darbepoetin alfa SC during the 3 week run-in period. Those previously treated with rHuEpo were converted using the equation: 200 IU rHuEpo = 1 μg darbepoetin alfa. During the run-in period, darbepoetin alfa doses were adjusted once weekly, based on weekly haemoglobin, to the next higher or lower unit dose as described below. At the end of the run-in period, the patients were randomized to either SC or IV darbepoetin alfa administration once weekly for 20 weeks. After these 20 weeks, the patients were switched to the alternative way of administration for the following 20 weeks.

The primary endpoint was the mean dose of darbepoetin alfa necessary to maintain the haemoglobin level in the defined range.

The patient demographics were registered at baseline after the run-in period, including weekly dose of darbepoetin alfa, haemoglobin, erythrocyte volume fraction (EVF), reticulocyte count, ferritin, transferrin, iron, TSAT, C-reactive protein (CRP), PTH, albumin and fractional urea clearance calculated as eKt/V. Darbepoetin alfa dose, haemoglobin, EVF and reticulocyte count were registered at weeks 0, 2, 4, 8, 12, 16, 17, 18, 19, 20, 22, 24, 28, 32, 36, 37, 38, 39 and 40. The frequent sampling during the first weeks of each interval, IV or SC, aimed to secure proper adjustment of darbepoetin alfa. In the last week of each interval, the frequent sampling was performed to mimic the sampling and loss of blood during run-in. Serum levels of ferritin, transferrin, iron, TSAT, CRP, PTH and albumin were registered at weeks 0, 4, 12, 20, 24, 32 and 40. Kt/V was measured at weeks 0, 12, 24 and 36.

Darbepoetin alfa was given once weekly and was available in unit doses of 10, 15, 20, 30, 40, 50, 60, 80, 100 and 150 μg. Darbepoetin alfa dose was adjusted to maintain each patient’s haemoglobin concentration within a target range of −0.8 to +0.8 mmol/l of the mean baseline haemoglobin and between 6.8 and 8.5 mmol/l throughout the study period.

In the event of a haemoglobin concentration below each patient’s individual target range or <6.8 mmol/l on two consecutive measurements, the dose of darbepoetin alfa was increased to the next higher unit-dose. In the event of a haemoglobin concentration above each patient’s individual target range or >8.5 mmol/l on two consecutive measurements, the dose of darbepoetin alfa was decreased to the next lower unit-dose.

IV iron supplementation was administered in order to maintain serum ferritin ≥300 μg/l. Since darbepoetin alfa was already registered for treatment of renal anaemia and was used as routine treatment in the unit, safety parameters and adverse events were not registered.

In the study design, we used the UCLA Department of Statistics ‘Normal Power Calculations’, available at http://calculators.stat.ucla.edu/powercalc/normal/n-1/n-1-samp.php. We assumed a 15% difference in ratio IV/SC to be clinically relevant and used 0.32 as standard deviation, based on our in-centre experience from a previous darbepoetin alfa study. Significance level 0.05, a two-tailed test and a power of 90% then required 50 patients to be available for the endpoint evaluation.

Mean dose of darbepoetin alfa in the ‘SC first’ and ‘IV first’ arms were compared using a paired, ratio two-tailed t-test. The paired t-test analyses the differences between pairs, but with data on rHuEpo or darbepoetin alfa dose there is very large variability among the differences. The differences are larger when the control value is larger. With such data, more consistent results are obtained when the ratio (treated/control) rather than the difference (treated − control) is used in the analysis. However, analysing ratios can lead to problems, because ratios are intrinsically asymmetric – all decreases are expressed as ratios between zero and one; all increases are expressed as ratios greater than 1.0. The traditional mathematical solution is to use the logarithm of ratios. With no change as zero, increases are then positive while decreases are negative. The 95% confidence interval (95% CI) of the difference is first computed as the 95% CI of the log (ratio) and then converted to the antilog of each end of the interval, thereby obtaining the 95% CI of the ratio [6]. Repeated analysis of variance (ANOVA) with post-test for linear trend was used to evaluate whether or not the protocol changes in darbepoetin alfa dose resulted in the intended stability of haemoglobin levels during IV or SC treatment in each period of the study arms. Also, ANOVA with post-test for linear trend was used to evaluate whether or not darbepoetin alfa dose was constant throughout both study periods (IV or SC), in both ‘SC first’ and ‘IV first’ study arms [6]. All analyses used a significance level of 0.05 and the two-tailed test. Traditional descriptive statistics was used to evaluate the patients in the two study arms, based on data from baseline and the run-in period. Analysis of covariance was applied to test whether or not haemoglobin concentration, PTH, ferritin or CRP measured at baseline were confounders for changes in the darbepoetin alfa dose during the study.

Results

A total of 71 patients were enrolled in the study. Two patients were withdrawn before randomization and 69 patients were randomized (Table 1). Of these,
11 were withdrawn during the study, five while they were treated with SC darbepoetin alfa and six on IV. The 13 patients not available for the endpoint evaluation were excluded due to withdrawal of consent (n = 3), kidney transplantation (n = 3), death (n = 6) and one was transferred to peritoneal dialysis. Out of the total of six patients who died, two were allocated to the SC first study arm, where one female patient aged 41 years was found dead at home in the first study week, while the other was a 74-year-old male who died at home during run-in: suicide was suspected. Four patients were allocated to the IV first study arm: a female aged 66 died in the 30th study week due to a cerebral infarction. One female aged 74 died in the 16th study week due to an acute myocardial infarction. A male patient aged 79 had a cardiac arrest in the 16th study week, following bilateral femoral amputation, and another male patient aged 84 was found dead at home in the 12th study week. Thus, 58 patients were available for endpoint statistical analysis.

Haemoglobin levels
For each patient, average mean haemoglobin was calculated from the area under the curve for all haemoglobin measurements performed. During the periods where the patients were given IV darbepoetin alfa, their mean haemoglobin was 7.62 mmol/l, as compared with mean haemoglobin during the SC darbepoetin alfa period of 7.67 mmol/l. The mean difference IV – SC was 0.051 mmol/l (95% CI: −0.132 to 0.236 mmol/l; paired t-test, P = 0.575; Figure 1). Thus, the difference was neither clinically nor statistically significant.

Since the study protocol intended to stabilize haemoglobin by regulating darbepoetin alfa dosage, the haemoglobin levels during the study periods were analysed further. During SC treatment in the ‘SC first’ arm of the study, three time intervals were compared with repeated measures ANOVA and post-test for linear trend. These intervals were weeks 1–4, weeks 8–12 and weeks 17–20 (ANOVA P = 0.307, NS; post-test for linear trend P = 0.137, NS). Likewise, the corresponding three intervals were compared during SC treatment in the ‘IV first’ arm (ANOVA P = 0.860, NS post-test for linear trend P = 0.672, NS). The same was tested during IV treatment in the ‘IV first’ arm (ANOVA P = 0.795, NS; post-test for linear trend P = 0.913, NS) and in the ‘SC first’ arm (ANOVA P = 0.143, NS; post-test for linear trend P = 0.065, NS). Thus, both IV and SC treatment resulted in stable haemoglobin levels with the darbepoetin alfa dose adjustments described in the protocol.

No blood transfusions were given during the study.

Darbepoetin alfa dose
Since the primary endpoint was a comparison of the ratio between SC and IV darbepoetin alfa doses, based on each patient’s 20 week mean darbepoetin alfa dose during SC and IV treatment, respectively, we first analysed the assumption that darbepoetin alfa dose was constant during SC or IV treatment. In each of the two treatment arms and during both SC and IV darbepoetin alfa treatment, three intervals were compared with ANOVA and post-test for linear trend. These intervals were weeks 1–4, weeks 8–12 and weeks 17–20. None of them showed significant trends: during SC treatment in the ‘SC first’ arm, repeated measures ANOVA showed P = 0.325 (NS), post-test for linear trend P = 0.142 (NS); during SC treatment in the ‘IV first’ arm, ANOVA P = 0.741 (NS), post-test for linear trend P = 0.442 (NS); during IV treatment in the ‘IV first’ arm, repeated measures ANOVA showed P = 0.345 (NS), post-test for linear trend P = 0.593 (NS); and during IV treatment in the ‘SC first’ arm,
repeated measures ANOVA showed $P=0.754$ (NS), post-test for linear trend $P=0.551$ (NS). Therefore, there was no statistical support for a possible carry-over effect of the switches from IV to SC or from SC to IV.

There was a large variability in the darbepoetin alfa doses used for IV or SC treatment of individual patients. Also, a large variability among the differences between SC and IV darbepoetin alfa dose was seen. Naturally, the difference between SC and IV dose was larger when the dose was high. Therefore, to get more consistent results, the protocol called for evaluation of the logarithm of the ratio IV/SC, rather than absolute values. The logarithm of ratios was used because ratios are intrinsically asymmetric [7].

Mean darbepoetin alfa dose during IV treatment was 32.1 µg (95% CI: 24.0–40.3), compared with a mean value for SC treatment of 34.1 µg (95% CI: 28.0–40.2). The ratio paired two-tailed $t$-test showed $P=0.036$, with a mean of the log(ratio) difference of 0.075 (95% CI: 0.005–0.146; Figure 2). Thus, it is unlikely that there is no difference in dose requirement of IV as compared with SC darbepoetin alfa. The CI of the ratio after antilog transform showed a 95% probability of a mean dose reduction between 1.2% and 28% by IV treatment instead of SC (Figure 2).

Analyses of covariance showed that haemoglobin concentration, eKt/V, PTH, ferritin or CRP measured at baseline were not statistically significant confounders for the darbepoetin alfa dose used during the study.

CRP, reticulocyte count, albumin or Kt/V did not change during the study intervals.

The amount of IV iron supplementation during each 20 week period was 864 ± 654 mg in SC treatment as compared with 833 ± 687 mg during IV treatment (NS). No blood was given. Ferritin during the SC periods increased slightly from 451 ± 241 to 512 ± 207 µg/l ($P=0.06$, NS) and during the IV periods from 454 ± 208 to 486 ± 228 µg/l (NS).

PTH was not stable, since it increased from 18 ± 22 to 21 ± 25 pmol/l ($P=0.046$) during SC treatment and from 17 ± 20 to 22 ± 25 pmol/l ($P=0.046$) during IV treatment. The difference between the SC and IV periods was not significant. There was no correlation between these changes in PTH and the darbepoetin alfa doses.

The power of the investigation was less than expected. From the observed values for the standard deviation (0.2688), the difference between means (0.05), the sample size of 58 and a significance level of 0.05 (two-tailed), the power was 56%.

**Discussion**

This single-centre prospective, randomized crossover study in a group of haemodialysis patients showed that a stable haemoglobin concentration could be maintained with a lower dose of darbepoetin alfa given by the IV as compared with the SC route. The statistical analysis indicated a 95% probability of a mean dose reduction between 1.2% and 28% by IV treatment instead of SC (Figure 2). However, it should be noted that this difference was found in a relatively small and selected group of stable patients in the maintenance phase of anaemia treatment. Therefore, further studies will be necessary to elucidate this finding in other types of patients and to narrow the CI and better quantify the economic impact of using IV rather than SC darbepoetin alfa.

The reasons for using a crossover design in the present study were 2-fold. Firstly, the individual patient served as their own control, which is rational in studies where the inter-patient variation is large and the expected intra-patient variation is lower. Secondly, when each patient serves as both treated and control, or self-control, the total number of patients needed to be included in a given study is halved and further reduced if, in fact, a lower variability is obtained in the measure of effect [8]. However, one pitfall might be a possible carry-over effect at crossover. But since the study drug was the same in the IV and in the SC period, a carry-over effect would indicate that there was, indeed, a difference in efficacy related to the route of administration. Given a close control of haemoglobin and sufficient darbepoetin alfa dose adjustments, a possible difference of efficacy should change the mean dose of darbepoetin alfa used; also the primary endpoint. Therefore, this study was designed to secure a tight control of the haemoglobin of the individual patient, especially around the crossover point. The data analysis showed this attempt to succeed, as there was no difference between mean haemoglobin during IV or SC darbepoetin alfa (Figure 1) and since haemoglobin was stable during IV or SC in both arms of the study. Thus, a possible difference in efficacy related to the route of administration was relayed to the primary endpoint, the mean dose of darbepoetin alfa used.

 **PTH** increased slightly in all groups during the study. We gradually implemented international guidelines limiting the amount of calcium-based phosphate binders given to the patients during the study. While this may explain the increase of PTH, both IV and SC

![Fig. 2. Reduced darbepoetin alfa during IV treatment. Ratio $t$-test on paired data, two-tailed $P=0.036$. (Left) The logarithm of the ratio, with mean and 95% CI for the logarithm of the ratio. (Right) The 95% CI for the IV darbepoetin alfa dose in per cent of SC dose.](http://ndt.oxfordjournals.org/)

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groups increased in parallel and there was no correlation between these changes in PTH and the darbepoetin alfa doses.

Previous studies using other endpoints have cautiously not claimed to demonstrate a difference in dose efficacy between IV or SC darbepoetin alfa. Brunkhorst et al. [3] found in a multicentre, prospective study including 1502 dialysis patients who were switched from rHuEpo to darbepoetin alfa IV or SC, that administration of darbepoetin by both routes was associated with stable haemoglobin concentrations. While both routes of darbepoetin alfa administration increased haemoglobin as compared with the level at baseline, IV patients increased more than SC patients with no overlap between the 95% CI of the mean changes in haemoglobin. Irrespective of the route of administration, the mean weekly darbepoetin dose necessary to maintain haemoglobin had decreased [3]. During IV, this decrease was from 23.23 to 19.92 µg/week, while the reduction during SC was from 22.95 to 21.61 µg/week. However, no statistical analysis of the possible difference in mean dose reduction was published. Locatelli et al. [7] switched dialysis patients previously maintained on rHuEpo treatment to darbepoetin alfa, IV or SC, with a conversion formula of 200 IU rHuEpo = 1 µg darbepoetin. They observed a decrease in mean weekly IV darbepoetin alfa dose requirements from 25.2 µg/week at baseline to 21.5 µg/week (P < 0.004) during the evaluation period 21–24 weeks after the switch. In contrast, SC weekly dosage requirements increased during the study period (20.8 to 22.7 µg/week; P = 0.014). When they compared the IV/SC dose ratio during the evaluation period, no significant difference was seen (0.95; 95% CI: 0.78–1.14). However, patients receiving IV darbepoetin had increased their mean haemoglobin concentrations while haemoglobin remained unchanged in SC patients [7].

Recently, Cervelli et al. [9] published a randomized crossover study comparing IV and SC darbepoetin dosing efficiency in haemodialysis patients. They reported that patients achieved non-significantly higher haemoglobin from a non-significantly lower darbepoetin dose with IV administration, while the population-based weight normalized SC/IV dose ratio was 1.04 (95% CI: 0.97–1.11). However, only 24 patients were available for their final analysis. Retrospectively, a 3% advantage towards SC dosing was excluded with 95% confidence and the study was concluded to have an 80% power to exclude a 10% change in either direction of dosing efficiency. Thus, the statistically non-significant tendency in the study of Cervelli et al. [9] is confirmed by the present investigation.

IV administration is preferred by haemodialysis patients and concern regarding increased immunogenicity of therapeutic proteins injected SC rather than IV [10] may further motivate a shift from SC to IV darbepoetin alfa.

In conclusion, the present single-centre open-label, prospective and randomized crossover study in 71 haemodialysis patients showed that darbepoetin alfa dosed by the IV route is more effective than by the SC route of administration.

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References

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