Pharmacokinetic and Pharmacodynamic Properties of Intravenous Darbepoetin Alfa (KRN321) in Japanese Hemodialysis Patients

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This article describes the pharmacokinetics, pharmacodynamics of darbepoetin alfa, a novel erythropoietin analogue showing a long-lasting effect. In the single-dose segment of this study, hemodialysis patients were administered darbepoetin alfa intravenously in increasing doses ranging from 90–180 μg/body. In the repeated-dose segment, switching to once-weekly administration of darbepoetin alfa was attempted in 14 patients who had been receiving intravenous rHuEPO two or three times a week. The intravenous dose of darbepoetin alfa to be administered once weekly (10–60 μg/body) was titrated to maintain hemoglobin levels within ±1.0 g/dL of the individual mean baseline level as well as in the range of 9–12 g/dL for 28 weeks. When given as a single dose, darbepoetin alfa showed almost linear pharmacokinetics within the dose range of 90–180 μg, and the mean elimination half-life ranged from 45.37 to 48.67 hours and the total body clearance ranged from 52.69 to 64.07 mL/hour/body. When given as repeated doses for up to 28 weeks, almost no changes were observed in the pharmacokinetics of darbepoetin alfa, i.e., the mean elimination half-life and total body clearance rate were 33.14 hours and 76.90 mL/hour/body at Week 1, 39.13 hours and 83.89 mL/hour/body at Week 21, and 42.09 hours and 83.48 mL/hour/body at Week 28. Darbepoetin alfa was well tolerated and no antibodies against it were detected. The results suggest that less frequent intravenous administration of darbepoetin alfa can effectively maintain target hemoglobin levels safely in the treatment of renal anemia in hemodialysis patients.

Key words: darbepoetin alfa, pharmacokinetics, hemodialysis, renal anemia, less frequent intravenous administration

Introduction

Darbepoetin alfa (KRN321)1–6 is a hyper-glycosylated analogue of recombinant human erythropoietin-alpha (rHuEPO), which stimulates erythropoiesis by the same mechanism as the endogenous hormone. To create KRN321, two extra N-linked carbohydrate addition sites were introduced into the primary sequence of rHuEPO using site-directed mutagenesis. KRN321 has five N-linked carbohydrate chains (Fig. 1), whereas rHuEPO and the endogenous hormone have three5. The sialic acid-containing carbohydrate moieties of erythropoietin critically influence its serum half-life and in vivo activity5. KRN321 was designed to verify the hypothesis that an erythropoietin molecule having a higher content of sialic acid would have a longer half-life as compared to the naturally occurring erythropoietin. KRN321, which contains five N-linked carbohydrate chains5, was discovered as a result of creating many new molecules and conducting various experiments. The clinical studies conducted to date revealed that the blood half-life of intravenous KRN321 is longer than that of intravenous rHuEPO (25.3 vs. 8.5 hr), suggesting that KRN321 might possibly enable once-weekly administration10.

Previously, a low single dose (10–60 μg) study

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was conducted in Japan with hemodialysis patients. In the present study, we investigated the pharmacokinetic and pharmacodynamic properties of KRN321 administered intravenously both as a high single dose and as repeated doses to Japanese hemodialysis patients.

**Subjects and methods**

Both single and repeated dosing studies were conducted in compliance with the revised Helsinki Declaration, and the study protocols were approved by the Institutional Review Board of both participating medical institutions (Shitoto Clinic, Hamamatsu, Japan, Shitoto Clinic Arai, Hamamatsu, Japan). Informed consent was obtained from all subjects in writing.

1. **Study drug**

KRN321 was supplied by Kirin Brewery Co., Ltd. (Tokyo, Japan)

2. **Single-dose segment of the study**

KRN321 was administered at three dose levels (90, 120 and 180 µg/body) to the same patients at sufficient intervals as a preparatory step to the investigation to determine the possibility of reducing the frequency in repeated administration. Administration was performed in a stepwise and dose-escalating manner after confirming the safety at the previous dose level.

Patients (age: 48 to 75 years; weight: 35.5 to 58.5 kg) diagnosed as having chronic kidney disease were recruited at 3 dialysis centers. Patients who had been undergoing hemodialysis three times a week for at least 3 months and receiving stable intravenous rHuEPO therapy for at least 3 months were enrolled. Additional inclusion criteria included a mean baseline, an Hb level of 9.8–11.9 g/dL, and an adequate level of stored iron (transferrin saturation more than 20%). A total of 10 patients completed the entire study without any dropouts. These patients received 90, 120 and 180 µg/body of KRN321 in this sequence at intervals of 4 and 6 weeks. At each administration, blood samples (4 mL) were taken before dosing and at 0.5 and 1, 2, 5, 8, 12, 24, 48, 96, 168 and 336 hours after dosing for pharmacokinetic analysis. Additional blood samples were taken at 504 and 672 hours at doses of 120 and 180 µg/body. KRN321 was administered to each patient as an intravenous bolus dose through a venous access catheter immediately after the 4-hour hemodialysis.

To monitor the safety of intravenous KRN321, vital signs including blood pressure, pulse rate and body temperature were periodically measured, and laboratory tests including hematological and blood biochemical tests and electrocardiography were periodically performed. The occurrence of adverse events was also monitored. The presence/absence of anti-KRN321 antibody was determined by a radioimmune precipitation assay before and 2 and 4 weeks after dosing.

3. **Repeated-dose segment of the study**

This segment was performed in 14 chronic kidney disease patients on hemodialysis at a single hemodialysis center in a prospective and open-label manner. Clinically stable patients aged 20 to 80
years whose mean Hb level was in the range from 9.5 to 11.5 g/dL and who had been receiving hemodialysis three times a week for at least 3 months were recruited. For enrollment, the subjects were required to have been receiving stable intravenous rHuEPO therapy two or three times a week for at least 8 weeks prior to study entry. To ensure an adequate level of stored iron to support erythropoiesis, the subjects were required to have a serum ferritin level of ≥ 100 ng/mL or a transferrin saturation of ≥ 20% by iron supplementation.

The exclusion criteria included uncontrolled hypertension (diastolic blood pressure of >100 mmHg observed on one-third of the measuring occasions in the 16 weeks prior to study entry), congestive heart failure (New York Heart Association class III or IV), hematologic disorders that might cause anemia, systemic infections, malignant or inflammatory states, elevated blood aspartate aminotransferase or alanine aminotransferase levels exceeding twice the upper limit of the normal range or other disorders which might interfere with the response to KRN321. Pregnant or lactating females were excluded. Patients who had received a blood transfusion or anabolic steroids, or had undergone a surgical operation associated with marked bleeding within the 16 weeks prior to study entry were also excluded. Finally, the physicians in charge were requested to make a clinical judgment on whether or not to exclude a potentially unsuitable patient based on any underlying disease.

In the 4-week screening and observation period, patients whose Hb and ferritin levels or transferrin saturation deviated from the aforementioned inclusion criteria were excluded. In the remaining eligible patients, rHuEPO which had been administered two or 3 times a week was switched to KRN321 administered once weekly after washout of rHuEPO for one week.

Since 1 µg of KRN321 is equivalent to 200–240 IU of rHuEPO on a protein mass basis, the initial dose level of KRN321 in each patient was determined assuming that twice-weekly administration of 1500 IU of rHuEPO would be equivalent to once-weekly administration of 15 µg of KRN321, and three doses of 1500 IU of rHuEPO per week would be equivalent to once-weekly administration of 20 µg of KRN321. Similarly twice-weekly administration of 3000 IU of rHuEPO would be equivalent to once-weekly administration of 30 µg of KRN321 and three doses of 3000 IU of rHuEPO per week would be equivalent to once-weekly administration of 40 µg of KRN321. The study period was set at 28–31 weeks from the first dose of KRN321 based on a previous clinical trial[30]. KRN321 was administered to each patient as an intravenous bolus dose through a venous access catheter immediately after the 4-hour hemodialysis. The dose level of KRN321 was thereafter adjusted by titrating within the range of 10–60 µg/body (10, 15, 20, 30, 40, 50 and 60 µg/body) to maintain the Hb level within the target ranges (baseline level ±1.0 g/dL as well as within the range from 9.0–12.0 g/dL) by measuring Hb level in each patient every 2 weeks, since the maintenance of Hb level was the primary efficacy endpoint. If the patient’s Hb level exceeded the target range on two consecutive measuring occasions, the dose level of KRN321 was decreased to the next lower level. On the other hand, if the patient’s Hb level was lower than the target range on two consecutive measuring occasions, the dose level of KRN321 was increased to the next higher level. Any change in dose level was limited to one-level up or down. If subjects treated with the highest dose level of KRN321 could not maintain the target Hb level, KRN321 was judged ineffective with this dosage regimen and such subjects were to be withdrawn from the study.

Blood samples (4 mL) for determination of pharmacokinetic parameters were taken at Week 1, Weeks 20–23 and Weeks 28–31. On each occasion, blood samples were taken before dosing and at 0.5 and 1, 2, 5, 8, 12, 24, 48, 96 and 168 hours after dosing with KRN321. Blood samples were also taken immediately before dosing at Weeks 1, 5, 9, 13, 17, 25 and 29 to determine the trough serum levels.

To monitor the safety of KRN321 given as repeated intravenous doses, vital sign measurements and laboratory tests were performed periodically. The occurrence of adverse events was also monitored. The presence/absence of anti-KRN321 antibody was determined by a radioimmuno precipitation assay before the first dose of KRN321 and when the treatment with KRN321 finished.

4. Pharmacokinetic analyses

Serum samples were analyzed using the Quantikine in vitro diagnostics rHuEPO enzyme-linked
Table 1  Patient demographics and baseline characteristics in the single-dose segment of the study

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
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<tbody>
<tr>
<td>Number of subjects</td>
<td>10</td>
</tr>
<tr>
<td>Number of Females</td>
<td>7 (70%)</td>
</tr>
<tr>
<td>Number of Males</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>(range)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>(range)</td>
</tr>
<tr>
<td>Cause of renal failure</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>6 (60%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>3 (30%)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Time since first dialysis (months)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>(range)</td>
</tr>
<tr>
<td>Baseline Hb (g/dL)</td>
<td>Mean±SD</td>
</tr>
<tr>
<td></td>
<td>(range)</td>
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</table>

The standard curve ranged from 0.078 to 5 ng KRN321/mL, and quality controls ensured individual assay quality. In validation tests, the intra-assay and inter-assay precision for spiked samples ranged from 5.1% to 11.0% and 4.4% to 6.1%, respectively. The interassay precision criterion for clinical serum samples was within 20%. The serum KRN321 concentrations were corrected for the concentrations of endogenous erythropoietin and rHuEPO (both show a cross-reaction in this assay) by direct subtraction.

The elimination half-life (t_{1/2}), systemic clearance (CL), volume of distribution at the steady state (Vss), initial volume of distribution (V0), mean residence time (MRT) and area under the serum concentration-time curve (AUC) were calculated by conventional non-compartmental methods using WinNonlin (version 3.3, Pharsight Corp., Mountain View, CA). In the single-dose segment of the study, the AUC from time zero to infinity was calculated at all dosage levels. In the repeated-dose segment of the study, the AUC from time zero to infinity was calculated at Week 1, and the AUC from zero to last sampling time was calculated at Weeks 20–23 and Weeks 28–31. The dose-normalized AUC (nAUC) was also calculated in the repeated-dose segment.

Results

1. Single-dose segment of the study

The patient demographics and baseline characteristics at study entry are shown in Table 1. All of the patients completed the procedures specified in the study protocol for both the first and second phases and were included in the pharmacokinetic and safety analyses.

1-1) Safety

KRN321 was well tolerated in this segment. All of the adverse events noted were those typically observed in the dialysis population, and none, except for one case, was definitely attributable to the treatment with KRN321. The exception was that obstruction of the arteriovenous fistula was noted in a patient after receiving 180 μg of KRN321.

Antibodies against KRN321 were not detected in any patient.

1-2) Pharmacokinetics

KRN321 disappeared from the serum in a bi-phasic manner: the time courses are shown in Figure 2 and the pharmacokinetic parameters are summarized in Table 2. After the single dose, the CL of KRN321 was in the range of 52.69 to 64.07 mL/hr/body. This slow CL resulted in an extended half-life of KRN321 (45.37-48.67 hr) as compared with that of rHuEPO. Since KRN321 is a large
molecule protein, the volume of distribution at the steady state was small (2785 to 2962 mL/body), indicating limited extravascular distribution of this molecule.

1-3) Pharmacodynamics

Figure 3 shows the time-course changes in Hb level after the higher single doses (90–180 µg) of KR323. The Hb levels increased gradually after dosing, peaking 264 hours after dosing and thereafter remaining between 10 and 11 g/dL for the 4-week monitoring period.

2. Repeated-dose segment of the study

Fourteen patients were enrolled in this segment of the study. The baseline patient characteristics are shown in Table 3. The anamnesis (cardiac infarction) and the complications present at the baseline (hypertension, diabetes mellitus, peripheral vascular diseases and coronary artery diseases) are commonly seen in this patient population. The diseases leading to chronic kidney disease were chronic glomerulonephritis, diabetes mellitus and hypertension. One patient was withdrawn from the study at Week 18 since his Hb level did not reach the target range in spite of the KR323 dose level being increased to 60 µg following the dose-titrating criteria. Another patient was withdrawn from the study at Week 14 and transferred to a larger hospital for surgery for lung cell carcinoma.

2-1) Safety

KR323 was also well tolerated in the repeated-dose segment of the study. All the adverse events noted were typical of the dialysis population and none were definitely attributable to the KR323.
Table 3  Patient demographics and baseline characteristics in the repeated-dose segment of the study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>14</td>
</tr>
<tr>
<td>Number of females</td>
<td>8 (57.1%)</td>
</tr>
<tr>
<td>Number of males</td>
<td>6 (42.9%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>Mean±SD 62.2±11.5 (range 39-70)</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>Mean±SD 52.0±10.16 (range 36.9-66.7)</td>
</tr>
<tr>
<td>Cause of renal failure</td>
<td></td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>11 (78.6%)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1 (7.1%)</td>
</tr>
<tr>
<td>Other</td>
<td>2 (14.3%)</td>
</tr>
<tr>
<td>Time since first dialysis (month)</td>
<td>Mean±SD 66.0±69.6 (range 14-267)</td>
</tr>
<tr>
<td>Baseline Hb (g/dL)</td>
<td>Mean±SD 9.9±0.59 (range 8.9-10.8)</td>
</tr>
</tbody>
</table>

The most commonly occurring adverse events were gastrointestinal symptoms such as diarrhea, dermatological disorders such as rashes and upper respiratory tract infections. In subgroup analysis of the adverse event profiles, in terms of age, gender, dialysis modality, baseline Hb level etc., there were no noteworthy differences in incidence among the subgroups related to any of these factors.

Over the entire study period, no relevant changes were observed in the mean systolic and diastolic blood pressure values, and there were no clinically relevant changes in usage of anti-hypertensive medications throughout the study period. There were no changes in laboratory parameters possibly associated with the treatment with KRN321. Each patient’s iron store status was stable throughout the study period. The mean serum ferritin concentration was 190.5 ng/mL at the baseline and it remained above 100 ng/mL throughout the study period (114.8-202.6 ng/mL).

There were no clinically meaningful changes in laboratory values. No antibody formation against KRN321 was detected in any patient after start of the study.

2-2) Pharmacokinetics

The time-course curves for the mean serum concentrations of KRN321 at Weeks 1, 21 and 28 showed a biphasic pattern as in the single-dose segment of the study. The dose-normalized pharmacokinetic profiles of KRN321 up to 168 hours after dosing were similar at Weeks 1, 21 and 28 (Fig. 4). The dose-normalized serum trough concentrations of KRN321, determined after the fifth dose did not change significantly for the remainder of the study (Fig. 5) suggesting that the steady state was achieved after the fifth dose of once-weekly administration of KRN321. There was no abnormal accumulation of KRN321 in the
The pharmacokinetic parameters are summarized in Table 4. A total of 12 patients (two patients were withdrawn from the study) were included in analysis. After the first dose (Week 1), the half-life and CL of KRN321 were 33.14 hr and 76.90 mL/hr/body, respectively, which were comparable to those observed in the single-dose segment of the study. No marked changes were observed in the CL or t_{1/2} after repeated administration (Table 4).

2-3) Pharmacodynamics

The mean Hb level was maintained around 10.5 g/dL throughout most of the study period and only one subject failed to maintain Hb concentrations within the target ranges. Mean Hb levels throughout the repeated-dose segment of the study are shown in Figure 6. There was a marked increase in Hb level during the initial 12-week period of the study, which was followed by a stabilization period extending over Weeks 12-20. A slight decline in Hb level was observed from Week 21 onward. The mean KRN321 dose level was exactly the same throughout the study period: 23.3 μg at Weeks 1, 21 and 28.

**Discussion**

The present study demonstrates the longer biological half-life, i.e., 45.37-48.67 hr, of KRN321 than rHuEPO, the half-life of which has been reported to be 4-12 hr with a mono-exponential decay following intravenous administration. Pharmacokinetic evaluations have revealed the almost linear property of KRN321. On the other hand, MacDougall et al reported that the serum half-life of KRN321 was 25.3 hr after a single dosing.

KRN321 effectively and safely maintained Hb concentrations between 10 and 11 g/dL based on Japanese guidelines. Moreover, Allon et al...
described that the half-life was 17.8, 23.4 and 23.6 hr at weeks 1, 12 and 36-40, respectively, in association with multiple dosings. These values are comparable to the present ones after repeated dosing. Although the underlying mechanism is not known, it has been indicated that the sialic acid-containing carbohydrate moieties of erythropoietin have a significant effect on systemic clearance, and that serum clearance is the primary determinant of in vivo biological activity\textsuperscript{15,16}. It is believed that increasing the sialic acid-containing carbohydrate of erythropoietin decreases serum clearance, thereby increasing the elimination half-life and in vivo activity. However, the stimulation of erythropoiesis does not only depend on an ambient circulating level of erythropoietin, but also on the mechanisms underlying the interaction of the hormone with its receptor. As yet it is unclear how long the molecule remains in contact with its receptor, and what kind of subsequent events occur by turning the switch on. It is reported that there are only approximately 200 erythropoietin receptors on each erythroid progenitor cell, with a maximum of 1000 per cell. This is in marked contrast to such other cell surface receptors as adrenergic receptors. There are also high-and low-affinity erythropoietin receptors. In addition, only 20-30% receptor occupancy is sufficient for stimulation of erythropoiesis, and it is speculated that there may be redundant erythropoietin receptors\textsuperscript{17}. The lack of information with regard to this biological process means that it is difficult to predict the optimum frequency of administration for an erythropoietic agent.

Interestingly, the comparative studies on the maintenance of Hb concentrations in renal anaemia patients on rHuEPO and less frequent KRN321 have shown apparent equivalence of intravenous and subcutaneous dosing requirements for KRN321. On the other hand the dose requirements of intravenous rHuEPO are known to be approximately 30% higher than for subcutaneous rHuEPO\textsuperscript{18}. It is considered that serum rHuEPO concentrations must remain above a threshold necessary for effective erythropoiesis. In subjects with normal renal function, this hypothetical threshold has been estimated to be between 30 and 200 mIU/mL of native hormone\textsuperscript{19}. In patients, serum concentrations of rHuEPO are initially high after intravenous administration, then rapidly decline, e.g., within 2 days, to below threshold levels. Therefore, on a three times per week intravenous regimen, the serum concentrations of rHuEPO might be inadequate to stimulate erythropoiesis for approximately one-fourth of each dosing interval. Following subcutaneous administration of rHuEPO, the slower absorption and more prolonged elimination of the drug may lead to a longer maintenance of serum rHuEPO concentrations above the threshold level until the next dosing day. As for intravenous KRN321, the longer elimination half-life may mean that serum concentrations of the drug remain above the threshold for longer, elucidating that the drug is equally efficacious, whether intravenous or subcutaneous administration.

The safety profile of KRN321 was comparable with that reported for rHuEPO. Adverse events were consistent with those expected for patients with chronic kidney disease undergoing dialysis and few events were considered to be related to KRN321 treatment.

Conclusions

In conclusion, the data from the present study confirm the efficacy and safety of KRN321 as an erythropoietic agent. The terminal half-life of KRN321 was longer than that of rHuEPO and the pharmacokinetics of KRN321 did not change as a function of time or dose, despite less frequent dosing compared with rHuEPO.

Moreover, Hb concentrations with each dose gradually increased and were maintained between 10 and 11 g/dL for a month after higher single-dose (90-180 µg) intravenous administration KRN321. Therefore, as single administration intravenous KRN321 maintains Hb concentration for a month or more, KRN321 is expected to expand the range of anemia therapy.

Acknowledgements

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