Pharmacokinetics and Pharmacodynamics of Intravenous and Subcutaneous Continuous Erythropoietin Receptor Activator (C.E.R.A.) in Patients with Chronic Kidney Disease

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Continuous Erythropoietin Receptor Activator (C.E.R.A.) is a new agent that is in development for the treatment of anemia with extended administration intervals in patients who have chronic kidney disease (CKD), both those on and those not on dialysis. This was an open-label, randomized, multicenter, two-period, crossover study in erythropoiesis-stimulating agent-naïve patients who had CKD and anemia and were receiving peritoneal dialysis. After a 1-wk run-in period, 16 patients were randomly assigned to receive a single administration of intravenous C.E.R.A. 0.4 μg/kg (n = 8) or subcutaneous C.E.R.A. 0.8 μg/kg (n = 8). Six weeks after the first administration of C.E.R.A. (4-wk assessment, 2-wk washout), the route of administration was switched so that all patients received single administrations of both intravenous C.E.R.A. 0.4 μg/kg and subcutaneous C.E.R.A. 0.8 μg/kg. C.E.R.A. had a prolonged and comparable half-life after intravenous (mean 134 h) and subcutaneous (mean 139 h) administration. Reticulocyte counts peaked at a median of 8 d after intravenous and subcutaneous administration with no difference in the time course between administration routes. This resulted in similar mean values for the area under the reticulocyte count-time curve (1191 × 10⁹ and 1193 × 10⁹/d L, respectively) and the maximum absolute increase in reticulocyte counts (36 × 10⁹ and 41 × 10⁹/L, respectively). C.E.R.A. has a prolonged and comparable half-life after intravenous or subcutaneous injection, suggesting that extended administration intervals may be feasible in patients with CKD.


Erythropoiesis-stimulating agents (ESA) have become a hallmark of anemia therapy since they first were introduced in the 1980s (1,2). Currently, three ESA are available for the treatment of renal anemia: epoetin α, epoetin β, and darbepoetin α.

The prevalence of end-stage renal disease (ESRD) is a problem that is increasing in magnitude (3). Despite the general success of ESA therapy for correcting anemia and maintaining hemoglobin (Hb) levels in patients with chronic kidney disease (CKD), the management of renal anemia with these agents often is labor-intensive and time-consuming, adding to the already escalating burden of care. Therefore, the development of a novel agent that corrects anemia effectively, maintains stable Hb levels, and allows less frequent administration may reduce the burden of anemia management for both patients and physicians.

Continuous Erythropoietin Receptor Activator (C.E.R.A.; Mircera®, Roche, Basel, Switzerland) is a new agent that is in development as a treatment for anemia with extended administration intervals in patients who have CKD and who are or who are not on dialysis. C.E.R.A. differs from erythropoietin through the integration of a large polymer chain that is linked via amide bonds between amino groups and methoxy polyethylene glycol-succinimidyl butanoic acid. The resulting molecule has a molecular weight of approximately 60,000 Da (4). Administration of comparable dosages of C.E.R.A. and epoetin β in animals resulted in higher reticulocyte counts with C.E.R.A., indicating superior potency in vivo (5). Studies in healthy subjects have shown that C.E.R.A. has a prolonged half-life (6,7). The pharmacokinetic properties of C.E.R.A., together with its receptor binding properties (8), give rise to a different pharmacologic profile compared with currently available ESA and suggest that extended administration intervals may be feasible. This is the first fully reported study of C.E.R.A. in patients with CKD, and its aim was to evaluate the pharmacokinetic and pharmacodynamic properties of C.E.R.A. after intravenous and subcutaneous administration in patients who receive peritoneal dialysis.

Materials and Methods

Patients

The study population consisted of ESA-naïve adult patients (≥18 yr of age) who had CKD and were receiving peritoneal dialysis. Patients were screened for eligibility during the 2 wk before the 1-wk study run-in period and entered the run-in period only when they met the

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following criteria: receiving peritoneal dialysis for at least 3 mo before screening, weekly Kt/V ≥1.8 and/or creatinine clearance >60 L/wk (as assessed within 3 mo of study start), and body weight ≤125 kg. To enter the first treatment period, patients were required to have Hb ≤12 g/dl and serum ferritin 50 to 1000 ng/ml. Values for Hb and serum ferritin were based on the mean of two assessments that were taken at screening and during the run-in period. Patients were excluded from the study for the following reasons: ESA therapy within previous 3 mo, significant surgery within previous 3 mo, blood transfusion within previous month or anticipated blood transfusion during 3 mo after study start, vitamin B₁₂ <200 pg/ml or serum folate <2.5 ng/ml, hemolysis (haptoglobin <30 mg/dl), bleeding necessitating treatment within previous 3 mo, hypertension (systolic BP >170 mmHg or diastolic BP ≥100 mmHg), illness or history of serious illness (e.g., severe cardiovascular or liver disease) within previous 3 mo, acute infection or inflammation, noncompliance with dialysis therapy, planned elective surgery during study period (except fistula surgery, which was considered essential for the management of these patients), albumin <3 g/dl, thrombocytes >500 × 10⁹/L, hyperparathyroidism, administration of other investigational drugs within 30 d of run-in period, known hypersensitivity to recombinant erythropoietin, and life expectancy <6 mo. All patients gave written informed consent. The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by local independent ethics committees.

**Study Design**

This was an open-label, randomized, multicenter, two-period, crossover study that was conducted in patients who were receiving peritoneal dialysis. After a 1-wk run-in period, patients were randomly assigned to one of two treatment sequences. At the beginning of the first treatment period, patients in group 1 received a single intravenous administration of C.E.R.A. 0.4 µg/kg, and patients in group 2 received a single subcutaneous administration of C.E.R.A. 0.8 µg/kg. The two dosages were selected on the basis of comparable reticulocyte responses and an absolute bioavailability for C.E.R.A. of approximately 50% after subcutaneous administration, observed in healthy volunteers (data on file). After 6 wk (4 wk of assessment followed by a 2-wk washout phase), the route of administration was switched so that each patient received a single administration of both intravenous C.E.R.A. 0.4 µg/kg and subcutaneous C.E.R.A. 0.8 µg/kg.

On the day of each C.E.R.A. administration, blood samples for pharmacokinetic assessments were taken before administration (intravenous and subcutaneous routes) and, for intravenous injections, 0.25, 2, 6, and 12 h after administration. Subsequent blood samples for pharmacokinetic analysis (intravenous and subcutaneous routes) were taken every day for the first 3 d after administration, then every 2 or 3 d up to 15 d after administration, with a final sample taken at the end of the 4-wk assessment period. Blood samples for pharmacodynamic assessments were taken on the day of each administration, then every 2 or 3 d up to 15 d after administration, with two additional samples taken at the end of the third and fourth weeks of the assessment period.

Blood samples for pharmacokinetic assessments were stored at room temperature for 30 min before centrifugation (1500 × g at 4°C for 10 to 15 min). After centrifugation, the supernatant was decanted into a clean plastic tube and stored at −20°C (or below) until analyzed.

Patients could withdraw from the study at any time and for any reason. In addition, patients could be withdrawn at the discretion of the investigator in the event of intermittent illness, adverse events, or protocol violation.

**Laboratory and Safety Assessments**

Serum C.E.R.A. concentrations were determined by ELISA using a primary mAb that was specific for C.E.R.A. and that did not cross-react with endogenous erythropoietin, and using a secondary polyclonal anti-lg antibody coupled to horseradish peroxidase. Reticulocyte counts were measured by flow cytometry using a Coulter Counter. Blood samples also were taken for regular laboratory safety tests (hematology and biochemistry). The presence of anti-C.E.R.A. antibodies was determined by a double-antigen bridging ELISA assay. Blood samples were tested for the presence of anti-C.E.R.A. antibodies during the run-in period and again on days 21 and 92. Adverse events were monitored throughout and graded according to severity. BP, heart rate, and electrocardiogram were also assessed at regular intervals during the study.

**Data Analyses**

Pharmacokinetic and pharmacodynamic analyses were performed using standard noncompartmental methods and actual sampling times. Pharmacokinetic parameters were calculated from serum C.E.R.A. concentration measurements. The primary pharmacokinetic parameters were clearance, maximum serum concentration (Cmax), elimination half-life (t₁/₂), area under the concentration-time curve from day 1 to the last measurement (AUC₁₋₂₁d), and bioavailability. The primary pharmacodynamic parameters were the area under the reticulocyte count-time curve over 20 d (AUE₁₋₂₀d) and the absolute maximum increase in reticulocyte count from baseline (AC-E_max). An ANOVA model with fixed factors “treatment” and “period” and the random factor “subject” was used to compare the pharmacodynamic parameters of intravenous C.E.R.A. 0.4 µg/kg and subcutaneous C.E.R.A. 0.8 µg/kg.

**Sample Size**

Initially, a sample size of 20 patients was planned. However, the study was terminated after 16 patients had completed treatment, because results indicated less variability in the data than expected. The sample size of 16 patients was sufficient to ensure the quality of the results and to address the objectives of the study.

**Results**

Sixteen patients who were receiving peritoneal dialysis were recruited and randomly assigned to treatment with C.E.R.A. All patients received single administrations of both intravenous C.E.R.A. 0.4 µg/kg and subcutaneous C.E.R.A. 0.8 µg/kg. Patient characteristics at baseline are summarized in Table 1. At baseline, five (31%) of the 16 patients had diabetes (one was insulin dependent, three were non–insulin dependent, and one was not classified). Fifteen (94%) patients received continuous ambulatory peritoneal dialysis, and one (6%) patient received intermittent automated peritoneal dialysis. There were no premature withdrawals, and all patients were included in the data analysis.

**Pharmacokinetics**

The mean serum C.E.R.A. concentration-time profiles after single administrations of intravenous C.E.R.A. 0.4 µg/kg and subcutaneous C.E.R.A. 0.8 µg/kg are shown in Figure 1A. The low clearance of C.E.R.A. observed with intravenous administration (0.494 ml/h per kg) resulted in a prolonged mean t₁/₂ of 134 h (Table 2). A similar mean t₁/₂ of 139 h was observed after subcutaneous administration of C.E.R.A. Using AUCₜₙₐₓₜ, the
The reticulocyte response to both intravenous C.E.R.A. 0.4 µg/kg and subcutaneous C.E.R.A. 0.8 µg/kg peaked at a median of 8 d after administration and then returned to levels that were close to baseline by day 21 (Figure 1B). At the dosages selected, there was no difference in the time course for reticulocyte counts between the two routes of administration, resulting in similar values for AUE1-21d and AC-E_{max} (Table 2). ANOVA of these pharmacodynamic parameters revealed that there was no significant difference between the two treatments (P = 0.678 for AUE1-21d and P = 0.606 for AC-E_{max}). Mean Hb concentration remained unchanged during the study after single administrations of intravenous C.E.R.A. 0.4 µg/kg and subcutaneous C.E.R.A. 0.8 µg/kg.

### Safety and Tolerability

C.E.R.A. was generally well tolerated, with the majority of adverse events being mild to moderate in intensity. Adverse events occurred with a similar frequency after intravenous and subcutaneous administration. The most frequent adverse events were headache (subcutaneous three of 16; intravenous two of 16) and vomiting (subcutaneous three of 16; intravenous zero of 16). In one patient, headache was experienced after both the first (intravenous 0.4 µg/kg) and second (subcutaneous 0.8 µg/kg) doses of C.E.R.A. All cases of headache and vomiting were of mild or moderate intensity and resolved during the study (headaches: four of six incidents resolved with appropriate treatment; vomiting: one of four incidents resolved with appropriate treatment).

### Pharmacodynamics

The reticulocyte response to both intravenous C.E.R.A. 0.4 µg/kg and subcutaneous C.E.R.A. 0.8 µg/kg peaked at a median of 8 d after administration and then returned to levels that were close to baseline by day 21 (Figure 1B). At the dosages selected, there was no difference in the time course for reticulocyte counts between the two routes of administration, resulting in similar values for AUE1-21d and AC-E_{max} (Table 2). ANOVA of these pharmacodynamic parameters revealed that there was no significant difference between the two treatments (P = 0.678 for AUE1-21d and P = 0.606 for AC-E_{max}). Mean Hb concentration remained unchanged during the study after single administrations of intravenous C.E.R.A. 0.4 µg/kg and subcutaneous C.E.R.A. 0.8 µg/kg.
appropriate treatment). Five serious adverse events (four after intravenous and one after subcutaneous administration) were reported in three patients. These events (exacerbation of chronic obstructive airways disease, myocardial infarction, hernia, catheter infection, and hyperglycemia) were not considered to be related to C.E.R.A. and resolved with appropriate treatment. No patients were withdrawn from the study as a result of adverse events. No anti-C.E.R.A. antibodies were detected in any of the patients. No patients required red blood cell transfusions during the course of the study.

Discussion

In patients who have CKD and receive peritoneal dialysis, single doses of C.E.R.A. administered either intravenously or subcutaneously elicited similar serum C.E.R.A. profiles with a prolonged serum half-life and low clearance. C.E.R.A. induced a sustained erythropoietic response after intravenous and subcutaneous administration. C.E.R.A. was generally tolerated, and no anti-C.E.R.A. antibodies were detected in any patient who participated in the study.

The calculated mean serum half-life of C.E.R.A. in peritoneal dialysis patients was 134 and 139 h after intravenous and subcutaneous administration, respectively. These results are consistent with the findings of studies in healthy subjects that reported a mean serum half-life for C.E.R.A. of approximately 130 h after intravenous and subcutaneous administration (6). The mean serum half-life of C.E.R.A., in both peritoneal dialysis patients and healthy subjects after intravenous or subcutaneous administration, is considerably longer than the half-life reported for epoetin α and epoetin β in healthy subjects (9) and also longer than the half-life reported for darbepoetin α in peritoneal dialysis patients (10) (Table 3). The clearance of C.E.R.A. in peritoneal dialysis patients after intravenous administration (0.494 ml/h per kg) was consistent with the clearance of intravenous C.E.R.A. that was observed in healthy subjects (0.372 ml/h per kg; data on file) and low compared with the clearance reported for other ESA that were administered intravenously (Table 3) (9,10). The bioavailability of C.E.R.A. in peritoneal dialysis patients after subcutaneous administration (52%) was consistent with previous findings in healthy subjects, which reported a bioavailability of 45 to 60% (7) and high compared with the reported bioavailability of other ESA that were administered subcutaneously (Table 3) (9,10). The explanation for the higher bioavailability of C.E.R.A. is not clear at this time.

C.E.R.A. demonstrated potent stimulation of erythropoiesis after a single intravenous or subcutaneous injection, with reticulocyte counts peaking approximately 8 d after administration. There was no difference in the reticulocyte response observed with subcutaneous C.E.R.A. 0.8 µg/kg and intravenous C.E.R.A. 0.4 µg/kg on the basis of AUE1-21d and AC-Emax data. From a pharmacologic perspective, the reticulocyte responses to single administrations of subcutaneous C.E.R.A. 0.8 µg/kg and intravenous C.E.R.A. 0.4 µg/kg do not necessarily predict the Hb response to these dosages of C.E.R.A. Therefore, these results should not be used to draw any conclusions regarding the dosages of C.E.R.A. that are required to achieve the same Hb response when administered intravenously or subcutaneously. Indeed, findings from clinical studies in dialysis patients have shown a similar Hb response to comparable C.E.R.A. dosages that were administered intravenously and subcutaneously (11,12). A similar reticulocyte response was observed in healthy volunteers, with reticulocyte counts peaking 7 to 10 d after intravenous and subcutaneous administration (6).

Normally, epoetin is internalized by erythroid progenitor cells and degraded after binding to the erythropoietin receptor. Results from our study are in accordance with those from preclinical studies reporting that C.E.R.A. acts differently at the receptor level, having a lower affinity for the erythropoietin receptor by associating significantly more slowly than epoetin (8). This may contribute to the distinct pharmacologic properties of C.E.R.A., including a prolonged half-life and extended administration intervals.

Conclusion

This study describes for the first time in patients with CKD the pharmacokinetic and pharmacodynamic properties of C.E.R.A. The data show a prolonged and comparable half-life for C.E.R.A. after intravenous and subcutaneous administration in this population. The extended half-life and low clearance of C.E.R.A. that were observed in this study result in a sustained production of reticulocytes. The pharmacokinetic characteristics of C.E.R.A. in patients with CKD suggest that extended administration intervals may be feasible, which po-

Table 3. Pharmacokinetic properties of erythropoiesis-stimulating agents a

<table>
<thead>
<tr>
<th>Agent</th>
<th>Administration Route</th>
<th>Mean (±SEM) Half-Life (h)</th>
<th>Mean (±SEM) CL (ml/h per kg)</th>
<th>F (%)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epoetin α b</td>
<td>Intravenous</td>
<td>6.8 ± 0.6</td>
<td>8.1 ± 0.2</td>
<td>100</td>
<td>Halstenson  et al. (9)</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td>19.4 ± 2.5</td>
<td>NA</td>
<td>31.9</td>
<td></td>
</tr>
<tr>
<td>Epoetin β b</td>
<td>Intravenous</td>
<td>8.8 ± 0.5</td>
<td>7.9 ± 0.3</td>
<td>100</td>
<td>Halstenson et al. (9)</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td>24.2 ± 2.6</td>
<td>NA</td>
<td>32.7</td>
<td></td>
</tr>
<tr>
<td>Darbepoetin α c</td>
<td>Intravenous</td>
<td>25.3 ± 2.2</td>
<td>1.6 ± 0.3</td>
<td>100</td>
<td>Macdougall et al. (10)</td>
</tr>
<tr>
<td></td>
<td>Subcutaneous</td>
<td>48.8 ± 5.2</td>
<td>NA</td>
<td>36.9</td>
<td></td>
</tr>
</tbody>
</table>

aNA, not available.

bStudy conducted in healthy subjects.

cStudy conducted in peritoneal dialysis patients.
tentially may reduce the workload of healthcare providers and offer greater convenience for patients. This is being evaluated further in phase III studies in patients with CKD.

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Responsibility for opinions, conclusions, and interpretation of data lies with the authors.

Disclosures

Dr. Macdougall has received research grants and honoraria from, and has acted as a scientific advisor, for F. Hoffmann-La Roche, Ortho Biotech, Amgen, and Affymax. Dr. Robson has performed contract research for F. Hoffmann-La Roche, GSK, MSD, Amgen, and a number of smaller companies, and has also served on an advisory board for Wyeth. Drs. Liogier, Pannier, Dougherty, Reigner, and Mr. Jordan are full-time employees of Roche, as indicated on the title page with the authors’ affiliations.

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