Comparison of the effects of weekly and biweekly intravenous CERA administration on erythropoiesis: A randomized controlled trial

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Abstract
Although continuous erythropoietin receptor activators (CERAs) are widely used erythropoiesis-stimulating agents for correcting renal anemia in patients undergoing hemodialysis (HD), few reports have examined weekly CERA administration. In this randomized controlled trial, we compared the efficacy and changes in the parameters of iron metabolism and erythropoiesis between weekly and biweekly CERA administration. In total, 120 patients undergoing maintenance HD were randomized to the weekly or biweekly group. The primary end point was the total CERA dose needed to maintain the target hemoglobin (Hb) levels during a 12-week evaluation period. There was no significant difference in the total dose between the weekly and biweekly groups (median 175.0 [interquartile range (IQR) 93.8–337.5] µg/12 weeks vs. 300.0 [IQR 125.0–375.0] µg/12 weeks, P = .18). The mean Hb levels during the evaluation period were 10.9 ± 0.8 g/dL in the weekly group and 10.7 ± 0.8 g/dL in the biweekly group (P = .25). Weekly CERA administration was well tolerated. Weekly CERA administration similarly managed anemia as biweekly administration in patients undergoing HD.

1 | INTRODUCTION

Anemia is a common complication that is associated with poor prognosis in patients undergoing hemodialysis (HD).¹,² The introduction of erythropoiesis-stimulating agents (ESAs) in the 1980s improved the prognosis and quality of life of patients requiring HD.³–⁵ Continuous erythropoietin receptor activators (CERAs) were launched in 2011 as long-acting ESAs in Japan. CERAs have a 5-fold longer half-life than recombinant human erythropoietin, an earlier ESA formulation, enabling less frequent administration.⁶,⁷ It has
been demonstrated that hemoglobin (Hb) levels can be maintained within the target range (10.0–13.5 g/dL) using biweekly or even monthly CERA administration, although the optimal interval has not been fully elucidated.

Some reports described the superiority of biweekly CERA administration over monthly administration in that biweekly administration could maintain Hb levels using a lower overall CERA dose. It is noteworthy that biweekly CERA administration maintained ferritin and hepcidin, a negative regulator of iron uptake in the small intestine and iron release from macrophages, at lower levels for 4 weeks after administration than monthly administration. This result suggested that biweekly administration is advantageous for absorption and iron utilization. Furthermore, we previously reported that ferritin and hepcidin declined to its lowest levels 1 week after monthly CERA administration. Therefore, weekly CERA therapy might result in improved iron utilization for erythropoiesis, thus leading to efficient Hb synthesis and reduced ESA requirements compared with conventional biweekly or monthly administration.

This study analyzed whether weekly CERA administration has advantages over biweekly CERA therapy regarding treatment efficacy (namely the dose required to maintain adequate Hb levels) and iron metabolism. To the best of our knowledge, this is the first randomized controlled trial to compare weekly and biweekly CERA administration.

2 | MATERIALS AND METHODS

2.1 | Participants

Outpatients who had been on maintenance HD for ≥12 weeks and treated with any ESA formulations for ≥12 weeks prior to the study were recruited during routine clinic visits through attending doctors at Kohsai Kai Kamioooka Jinsei Clinic and Kohsai Kai Bunkojin Clinic in Yokohama, Japan. The details of the inclusion and exclusion criteria are provided in Table 1. The study protocol was approved by the Yokohama City University Certified Institutional Review Board (approval number CRB18-001). The study was registered in the Japan Registry of Clinical Trials as jRCTs031180030. The study was conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

2.2 | Study design

This prospective, open-label, randomized, parallel-group clinical trial compared efficacy and iron metabolism between once-weekly (QW) and biweekly (Q2W) CERA administration in patients undergoing maintenance HD. This study period consisted of four parts (Figure 1): a 2-week baseline period (weeks −10 to −9), an 8-week titration period (weeks −8 to −1), a 12-week evaluation period (weeks 0–11), and a 4-week follow-up period (weeks 12–15). After the baseline period, patients were randomly assigned (1:1) to the CERA QW or Q2W group. Both groups received CERA biweekly during the titration period, and

### Table 1: Inclusion and exclusion criteria

| Inclusion criteria                                           |
|-------------------------------------------------------------|
| - Adult patients (≥20 years old) with renal anemia          |
| - Regular HD for at least 12 weeks                          |
| - ESA treatment for at least 12 weeks                       |
| - Provision of written informed consent                     |

| Exclusion                                                   |
|-------------------------------------------------------------|
| - Chronic congestive heart failure (New York Heart Association Class III or IV) |
| - Uncontrolled hypertension (diastolic BP ≥ 110 mm Hg before HD) |
| - Chemotherapy or radiation therapy for malignancy within 24 weeks |
| - Red blood cell transfusion within 12 weeks                 |
| - Open abdominal or chest surgery within 12 weeks            |
| - Pregnancy                                                  |
| - Those who are judged as ineligible for study for other reasons |

Abbreviations: BP, blood pressure; ESA, erythropoiesis-stimulating agent; HD, hemodialysis.

patients were administered the drug once-weekly or biweekly according to the group assignment during the evaluation period.

2.3 | CERA dose adjustment and administration

2.3.1 | Dose adjustments

The initiation CERA dose during the titration period (ie, the dose at week −8) was based on the ESA dose received during the preceding 2 or 4 weeks as follows. When the dose of recombinant human erythropoietin was ≥4500 units or <4500 units per week, 75 or 50 µg of CERA were administered, respectively. For darbepoetin alfa, the CERA dose was calculated as the darbepoetin total dose in the preceding 2 weeks (µg) × 1.2. For the CERA, the initial dose was calculated as half of the total CERA dose (µg) in the preceding 4 weeks.

During the titration and evaluation period, the CERA dose was adjusted to maintain Hb levels within the range of 10–11.5 g/dL in reference to the Guidelines for Renal Anemia in Chronic Kidney Disease published by the Japanese Society for Dialysis Therapy. Dose adjustments were performed every 2 weeks (starting in week −8) based on Hb levels as follows. The CERA dose was adjusted at seven levels: 0, 25, 50, 75, 100, 125, and 150 µg per 2 weeks (Figure 2A). The dose level for the next 2 weeks was based on the Hb value obtained 1 week before dose adjustment as indicated in Figure 2B.

2.4 | Administration

During the titration period, both the CERA QW and Q2W groups received the total prescribed CERA dose as determined in Figure 2 once every 2 weeks (weeks −8, −6, −4, and −2). During the evaluation period, the CERA QW group received the half the prescribed dose once
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a week, whereas the CERA Q2W group received the total amount every 2 weeks. During each administration, the CERA (Mircera, Chugai Pharmaceutical, Tokyo, Japan) was injected through the dialysis circuit immediately after the first dialysis session of the week.

2.5 | Discontinuation criteria

The study protocol for each patient was discontinued if any of the following events occurred: Hb ≤7.5 g/dL for three consecutive assessments despite the maximal CERA dose (150 µg per 2 weeks), a requirement for red blood cell transfusion, and loss to follow-up.

2.6 | Iron supplementation

Intravenous iron administration was initiated when ferritin levels fell below 100 ng/mL and continued on a weekly basis for a total of 13 injections unless serum ferritin levels exceeded 300 ng/mL. During each administration, 40 mg of elemental iron (Fesin, Nichi-Iko Pharmaceutical, Toyama City, Japan) were slowly injected via the dialysis circuit.

2.7 | Blood sampling and assessments

Blood samples were obtained before the HD session at the beginning of each week. Hb levels were measured every 2 weeks during the titration period and every week during the evaluation period. Iron metabolism markers (serum iron, total iron binding capacity, serum ferritin, and transferrin saturation [TSAT]), reticulocyte hemoglobin equivalent (Ret-He), and hepcidin levels were measured weekly from week 0 to week 2. HD was performed 3 times per week regularly for 3–6 hours with a dialysate flow rate of 400–500 mL/min, and the blood flow rate ranged from 120 to 300 mL/min. Hemodialysis/hemodiafiltration prescription was adjusted by attending doctors as necessary during the study period.
2.8 | Analytical methods

Serum hepcidin levels were quantified using liquid chromatography/tandem mass spectrometry. Ret-He describes the Hb content per reticulocyte, and it is a similarly valuable indicator as the reticulocyte hemoglobin content for evaluating iron utilization. Ret-He levels were quantified using an XE–5000 hematology analyzer (XE RET MASTER, Sysmex, Kobe, Japan). Although the Hb concentration is affected by multiple factors over approximately 3 months prior to the measurement, Ret-Hb is a specific and short-term indicator of newly synthesized Hb. The Ret-Hb level is a beneficial index for estimating the efficiency of erythropoiesis during the previous 3–4 days. Ret-Hb levels (mg/dL) were calculated by multiplying the Ret-He content (µg) per cell by the reticulocyte count (1 × 10⁹ cells/L) according to previous reports.

2.9 | Study outcomes

The primary end point was the total CERA dose administered during the evaluation period. The secondary end points were mean Hb levels during the evaluation period, and changes in parameters of iron metabolism and erythropoiesis from week 0 to week 2. Safety and tolerability were assessed according to adverse events (AEs) reported throughout the study period. An AE is any unfavorable and unintended sign, symptom, or disease observed during the study regardless of causality with treatment.

2.10 | Statistical analysis

We hypothesized that the difference of the CERA dose would be 25 ± 40 µg per 4 weeks based on a previous report. The number of patients required using a two-sided significance level of .05 and statistical power of 80% was calculated to be 41 in each group. We calculated the final target number of patients as 60 in each group to account for dropouts. Unless otherwise specified, values are presented as the mean ± SD or median and interquartile range (IQR). Differences between the two groups were analyzed using an unpaired t test for parametric variables or Wilcoxon’s rank-sum test for non-parametric variables. The Wilcoxon signed-rank test was used to compare the values at different weeks in the same group. Categorical data were compared using the chi-squared test or Fisher’s exact test. P < .05 was considered statistically significant. Statistical analyses were performed using SPSS for Windows 25.0 (SPSS, Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Patient disposition

Figure 3 presents a flow chart of this study. After the inclusion and exclusion assessment, 122 patients were enrolled, 120 of whom were randomly assigned to the QW (n = 60) or Q2W (n = 60) group. There were no significant differences between the two groups regarding the baseline characteristics excluding blood urea nitrogen (Table 2).

Among them, 57 patients each entered the evaluation period in the QW and Q2W groups. During the titration period, three patients in the QW group discontinued the study because of hospital admission (n = 2) and transfusion (n = 1), whereas three patients in Q2W group discontinued because of transfusion (n = 2) and clinic transfer (n = 1). During the evaluation period, three patients in the QW group discontinued the study because of hospital admission (n = 1) and clinic transfer (n = 2), whereas four patients in the Q2W group withdrew because of withdrawal of consent (n = 1) and hospital admission (n = 3). In total, 107 patients (QW group, n = 54; Q2W group, n = 53) completed the evaluation period. No significant differences in the baseline characteristics were detected between patients in the two groups who completed the evaluation period.

3.2 | The treatment efficacy

There was no significant difference in the total CERA dose during the evaluation period between the QW and Q2W groups (175.0 [IQR 93.8–337.5] vs. 300.0 [IQR 125.0–375.0] µg/12 weeks, P = .18; Figure 4). No difference was noted in proportion of patients who needed iron supplementation during the evaluation period between the QW and Q2W groups (40.7% vs. 34.0%, P = .47). The mean Hb levels during the evaluation period were 10.9 ± 0.8 g/dL in the QW group and 10.7 ± 0.8 g/dL in the Q2W group (P = .25). The median percentage of patients with Hb levels in the target range of 10.0–11.5 g/dL in the 12 blood samplings during the evaluation period was 41.7 (IQR 33.3–66.7) % in the QW group, versus 41.7 (IQR 25.0–58.3) % in the Q2W group (P = .93). Similarly, no difference was detected in the percentages of patients with Hb levels below (16.7 [IQR 0.0–43.8] % vs. 33.3 [IQR 0.0–50.0] %, P = .16) or above (25.0 [IQR 8.3–50.0] % vs. 16.7 [IQR 0.0–41.7] %, P = .13) the target Hb range during the evaluation period between the QW and Q2W groups.

3.3 | Changes in parameters of iron metabolism and erythropoiesis

Changes in the parameters of iron metabolism during 2 weeks of CERA administration are presented in Figure 5A-C. The change in ferritin levels in week 1 was significantly larger in the Q2W group than in the QW group (−13.8 [IQR −46.3–3.9] ng/mL vs. −5.2 [IQR −20.6–10.5] ng/mL, P = .029). The change in hepcidin levels in week 1 tended to be larger in the Q2W group than in the QW group (−10.2 [IQR −36.7–1.7] ng/mL vs. −4.4 [IQR −16.8–5.6] ng/mL, P = .068). The change in TSAT levels in week 1 was significantly larger in the Q2W group than in the QW group (−3.0 [IQR
−12.0−0.0] % vs. 0.0 [IQR −5.0–−5.3] %, \( P = .010 \). Conversely, there were no significant differences in these parameters between the groups in week 2.

Regarding the changes in the parameters of iron metabolism within each group during the first 2 weeks of administration, ferritin levels in the Q2W group were significantly lower in week 1 than in week 0 (167.4 [IQR 33.5–263.1] ng/mL vs. 200.6 [IQR 49.5–293.6] ng/mL, \( P = .002 \)), but no difference was noted between weeks 0 and 2 (192.4 [IQR 58.4–284.2] ng/mL, \( P = .11 \)). Similarly, hepcidin levels in the Q2W group were significantly lower in week 1 than in week 0 (37.2 [IQR 2.0–128.7] ng/mL vs. 79.7 [IQR 28.0–139.3] ng/mL, \( P = .005 \)), but no difference was noted in week 2 (98.8 [IQR 34.6–129.9] ng/mL, \( P = .17 \)). Moreover, TSAT levels in the Q2W group were significantly lower in week 1 than in week 0 (27.0 [IQR 15.5–39.0] % vs. 31.0 [IQR 24.0–43.0] %, \( P = .002 \)), but no difference was noted in week 2 versus week 0 (32.0 [IQR 19.5–43.0] %, \( P = .26 \)). Contrarily, no differences were noted in any of these parameters after 1 or 2 weeks of treatment relative to week 0 in the QW group.

Changes in the parameters of erythropoiesis are presented in Figure 5D–E. After 1 week of administration, the change in Ret-Hb levels was significantly larger in the Q2W group than in the QW group (24.0 [IQR −1.7–72.9] mg/dL vs. 1.3 [IQR −13.6–25.9] mg/dL, \( P = .001 \)). However, after 2 weeks, the change in Ret-Hb levels was significantly smaller in the Q2W group than in the QW group (−0.6 [IQR −12.3–13.2] mg/dL vs. 11.3 [IQR −9.0–46.9] mg/dL, \( P = .035 \)). Contrarily, the change in Ret-He levels was significantly larger in the Q2W group than in the QW group in week 1 (−0.8 [IQR −3.6–0.3] pg vs. −0.3 [IQR −1.2–0.4] pg, \( P = .011 \)).

### 3.4 Safety and tolerability

The overall incidences of all AEs were 92% (55/60) in the Q2W group and 97% (58/60) in the QW group (\( P = .44 \)). The AEs were consistent with those typically found in a previous study of patients undergoing HD. The frequently observed AEs included fluid overload (37%), upper respiratory tract infection (32%), vascular access complication (19%), hypotension (14%), hypertension (13%), dizziness (8%), muscle spasms, diarrhea, and nausea or vomiting (5%). There was no significant difference in the proportion of the patients required an increase in the antihypertensive agent dose between QW and Q2W group (8.3% vs 18.3%, \( P = .11 \)). The proportions of patients who experienced severe AEs were similar between the QW and Q2W groups (17% vs. 13%). There were no treatment-related AEs.
This is the first study to assess the efficacy and safety of weekly CERA administration in patients undergoing HD. The total CERA dose required to maintain the target Hb levels did not differ between weekly and biweekly administration. Although previous studies reported that biweekly CERA administration maintained Hb levels at a lower total dose than monthly administration, further shortening of the administration interval (weekly administration) did not reduce the total CERA dose in the present study.

Hepcidin is a key mediator of iron metabolism generated by the liver. Hepcidin regulates plasma iron levels by decreasing iron absorption and the release of recycled Hb iron and increasing intracellular iron stores. CERAs were previously reported to suppress hepcidin levels, inducing the release of stored iron and effective erythropoiesis. Unexpectedly, the changes in hepcidin levels did not differ between the weekly and biweekly groups.
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Similarly, ferritin and TSAT levels did not change in the QW group. The CERA dose per time might have been insufficient to decrease hepcidin levels in the QW group. Conversely, in the Q2W group, hepcidin, ferritin, and TSAT levels were significantly lower in week 1 than in week 0. In addition, the changes of Ret-Hb levels tended to contradict those of hepcidin, ferritin, and TSAT levels in both groups. Ret-Hb is considered an actual indicator reflecting functional iron availability for erythropoiesis. Although there were no significant differences in hepcidin, ferritin, and TSAT levels in week 2 between the Q2W and QW groups, these results potentially indicate that the promoting effect of iron utilization of biweekly CERA administration is better than that of weekly administration at a lower dose.

Considering effective renal anemia treatment in patients undergoing HD and the burden on medical staff, biweekly CERA administration may represent a more balanced regimen than weekly administration.

The results of the present study did not reveal significant differences in the mean Hb levels and the proportion of patients to achieve the target Hb range at the Q2W and QW groups, which enabled us to compare the CERA dose between the two groups as an index of treatment efficacy. Hence, our study had an appropriate environment for comparing treatment efficacy based on the CERA dose.

In the present study, the proportion of patients who achieved the target Hb range was relatively low compared to previous studies which compared biweekly and monthly administration of CERA. Although these previous studies had inclusion criteria for Hb range between 10.5 and 13.0 g/dL, our present study did not have inclusion...
criteria for Hb levels. Further, the previous studies set a wider target Hb range of 10.0–13.5 g/dL compared to the present study (10.0–11.5 g/dL). These differences probably led to low achievement of the target Hb range in the present study.

The AE profiles in the present study were consistent with previous reports in patients undergoing HD. Most AEs were mild to moderate in severity. We were concerned about an increase in the AE rate in the QW group because of the long half-life of the drug. However, AE rates did not differ between the groups, indicating that once-weekly CERA administration was well tolerated.

Several limitations of the present study must be considered. First, because our study did not focus on certain ferritin conditions such as iron deficiency or high ferritin levels, it is unclear whether the results can be applied to such conditions. Second, because this study did not focus on hyporesponsiveness to ESAs, it is unclear whether the results are applicable to those populations. Third, because Hb levels in both groups were well-controlled at baseline, it is difficult to justify whether weekly and biweekly administrations will be equally effective for patients with uncontrolled anemia. Fourth, because we measured parameters of iron metabolism and erythropoiesis over a short period, we cannot discuss their long-term changes.

In conclusion, this is the first randomized controlled trial to assess the efficacy on weekly CERA administration in patients undergoing HD. The total CERA dose needed to maintain Hb levels in the target range did not differ between weekly and biweekly administration.

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CONFLICT OF INTEREST
The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS
YK, YT, HW, TF, EU, HM, TK, TK, and KT designed and conducted the research. YK, YT, HW, TF, EU, KA, HM, TK, SY, TO, and KT collected the data. YK, HW, TF, and KA analyzed the data. YK, HW, TF, and EU wrote the manuscript. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The data used to support the findings of this study are included within the article.

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