Novel Compound Induces Erythropoietin Secretion through Liver Effects in Chronic Kidney Disease Patients and Healthy Volunteers

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Keywords

Anemia · Erythropoietin · Hypoxia-inducible factor-inhibitor · TP0463518

Abstract

Background: TP0463518 is a novel hypoxia-inducible factor prolyl hydroxylase inhibitor developed to aid in the treatment of anemia associated with chronic kidney disease (CKD) and is expected to increase erythropoietin (EPO) derived from liver. Two phase I studies were conducted in healthy volunteers (HV) and CKD patients undergoing hemodialysis (i.e., HD patients) or those not undergoing dialysis (i.e., ND patients). **Methods:** Pharmacokinetics, pharmacodynamics, and safety profiles of TP0463518 were assessed. Forty HV received single oral doses of TP0463518 at 3, 6, 11, 20, and 36 mg or placebo. Twenty ND patients received single doses of TP0463518 at 1, 6, and 11 mg and 9

HD patients received TP0463518 at 1 and 11 mg doses. To identify the source organ of EPO, glycosylation patterns were determined using percentage migrated isoform (PMI) values. **Results:** Declining renal function slowed elimination of TP0463518 and increased the mean $AUC_{0-\infty}$. ΔE_{max} of serum EPO in 11-mg groups of HV, ND patients, and HD patients were 24.37 ± 11.37 , 201.57 ± 130.34 , and $1,324.76 \pm 1,189.24$ mIU/mL respectively. A strong correlation was observed between logarithm conversions of ΔE_{max} and $AUC_{0-\infty}$ with correlation coefficients of 0.945. PMI values of blood after TP0463518 administration were elevated to similar or higher levels in comparison with those of umbilical cord blood, which mainly contains liver-derived EPO. **Conclusions:** TP0463518 induced dose-dependent EPO production, mainly derived from the liver in HV and CKD patients. These results suggest that TP0463518 is a new strategy for treating anemia in CKD, which can be used regardless of renal functions.

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Introduction

Anemia, a common complication of chronic kidney disease (CKD), has been associated with a lower quality of life and an increased risk of cardiovascular diseases and death [1, 2]. A deficiency in renal production of erythropoietin (EPO) is the main factor causing anemia [3, 4].

Hypoxia-inducible factor prolyl hydroxylase inhibitors (HIF-PHIs) are an emerging class of compounds for the treatment of renal anemia by stimulating endogenous EPO production [4, 5].

Kidneys are the dominant organ for EPO production in healthy adults, whereas EPO is mainly produced by the liver in fetuses and neonates [6, 7]. Because the ability to produce EPO is influenced by the extent of kidney disease, renal pathology, and remaining renal mass, a therapeutic strategy that targets EPO production in the liver may prove to be highly beneficial for CKD patients [5]. However, although at least 6 HIF-PHIs have been tested in humans to date, the origin of EPO by most HIF-PHIs in clinical development have not identified or clarified [5]. Only FG-2216 has been reported to increase EPO concentrations even in anephric patients and it has been indicated that certain organs other than the kidneys may contribute to the increase in EPO concentrations through HIF activation [8]. In our preclinical study on TP0463518, EPO production was induced in bilaterally nephrectomized rats, suggesting that the compound has the unique feature of inducing liver-specific EPO production [9].

Here, we report 2 single-dose phase I studies. We conducted the first study in healthy volunteers (the HV study) and assessed pharmacokinetics (PK), pharmacodynamics (PD), and preliminary safety of TP0463518. Referring to the results of the HV study, we designed and conducted the second study in CKD patients undergoing or not undergoing hemodialysis (the CKD study). In addition, we aimed to elucidate the source organ of EPO induced by TP0463518 in human.

Methods

The full version of inclusion and exclusion criteria, methods for determining concentration of TP0463518 in plasma and urine, and methods for EPO glycosylation pattern analysis were applicable in online supplementary Materials (for all online suppl. material, see www.karger.com/doi/10.1159/000492181).

Study Design

The HV study was a double-blinded, randomized, placebo-controlled, single-dose-escalation study conducted at a single center in Japan. The CKD study was an open-labeled, single-

dose-escalation study conducted at 9 medical institutions in Japan. Both studies were conducted in accordance with the Declaration of Helsinki, standards of the Japanese Pharmaceutical Affairs Law and Good Clinical Practice. Study protocols were reviewed and approved by the institutional review boards of all participating institutions. All subjects provided written informed consent.

Participants

HV Study

This study included Japanese adult men (20–40 years) and no significant medical conditions identified during screening tests. Key exclusion criteria were a hemoglobin (Hb) concentration ≥ 16.2 g/dL and serum EPO concentration outside reference ranges (4.2–23.7 mIU/mL).

CKD Study

This study included Japanese CKD patients aged 20–75. Key inclusion criteria for CKD patients not undergoing dialysis (i.e., ND patients) were Hb of 10.0–13.0 g/dL and an estimated glomerular filtration rate of 15–45 mL/min/1.73 m². Key inclusion criteria for CKD patients undergoing hemodialysis (i.e., HD patients) were Hb of 10.0–12.0 g/dL.

Interventions

HV Study

All subjects were admitted to the clinical unit on Day –1 (the day prior to administration), discharged at Day 4 (72 h post-dose), and they revisited at Follow-up 1 (Day 6) and Follow-up 2 (Day 8). Each subject received a single oral dose of TP0463518 (3, 6, 11, 20, or 36 mg) or matching doses of the placebo, taken in a fasting state. The starting dose was selected based on the no-observed-adverse-effect-level exposure from preclinical studies. The doses of subsequent steps were selected based on reviews of PK and PD and preliminary safety data from the previous step. For 11-mg TP0463518 and its placebo, administration following food consumption was tested in the same subjects.

CKD Study

All subjects were admitted to the clinical unit on Day –1, discharged at Day 2 (24 h post-dose), and they revisited at Follow-up 1 (Day 4) and Follow-up 2 (Day 7). For some HD patients, Follow-up 1 was conducted only on Day 5 according to their dialysis schedules. ND patients received a single oral dose of TP0463518 (1, 6, or 11 mg), whereas HD patients received a single dose of TP0463518 (1 or 11 mg). The lower starting dose was selected to secure the safety of patients because the plasma exposure of TP0463518 in the patients with renal impairment was likely to increase as compared to that in HV. The doses of subsequent steps were selected based on reviews of PK and PD and preliminary safety data from the previous step. The study drug was administered 30 min after breakfast. HD patients were administered the study drug on the day following dialysis. ND patients who received 1-mg TP0463518 were limited to CKD stage 3b (estimated glomerular filtration rate 30–45 mL/min/1.73 m²).

PK Assessments

Plasma concentrations of TP0463518 were determined from blood samples collected from pre-dose to 72 h post-dose (in the HV study) or Follow-up 2 (in the CKD study). Urine samples for

determining urine concentrations of TP0463518 were collected from 3 h before study drug administration to 72 h post-dose (in the HV study) or 24 h post-dose (in the CKD study).

PD Assessments

Blood samples for determining serum EPO concentrations were collected from pre-dose to 72 h post-dose (in the HV study) or Follow-up 1 (in the CKD study). Serum EPO concentration was determined by Chemiluminescent Enzyme Immuno Assay methods.

Safety Assessments

Safety evaluation was based on clinical adverse events (AEs), vital signs, 12-lead electrocardiograms, and clinical laboratory results reported during the study. AEs were studied from the start of study treatment until the Follow-up 2. Hematology assessments, including Hb concentration and reticulocyte counts, were performed pre-dose, 24, 48, 72 h after dosing, Follow-up 1 and 2 for the HV study and pre-dose, 24 h after dosing, and Follow-up 2 for the CKD study.

EPO Glycosylation Pattern Analysis

For EPO glycosylation pattern analysis, test plasma with the highest EPO concentration was chosen from samples taken 6, 8, and 12 h after administration. Glycosylation patterns were determined using percentage migrated isoform (PMI) values [10].

Statistical Analysis

PK parameters were calculated based on plasma TP0463518 concentrations by non-compartmental analyses using WinNonlin Phoenix version 7.0 (Certara, NJ, USA). In HD patients, PK parameters were calculated assuming that hemodialysis had no effect on drug elimination because of high protein binding of TP0463518 in human plasma (>99%, data not shown). The urinary excretion rates of TP0463518 in the 24 h following administration (Fe_{0-24h}) were calculated based on urinary concentrations of TP0463518 and urine volume.

Regarding PD parameters, E_{max} was defined as the maximum serum EPO concentrations observed during the study and ΔE_{max} was calculated as change from baseline values.

Data from the 2 studies were combined for analyzing the relationship between PD and PK parameters. Correlation coefficients between logarithmic conversions of ΔE_{max} and both PK parameters (C_{max} and $AUC_{0-\infty}$) were calculated.

Statistical analyses were conducted using SAS software (version 9.2 and 9.4; SAS Institute Inc.). Data are presented as mean $\pm$ SD, unless otherwise noted.

Results

Subject Disposition and Baseline Characteristics

After the screening, 40 HV, 20 ND patients, and 9 HD patients were enrolled in the respective studies. All participants completed the studies.

Mean Hb concentrations in CKD patients were 11.05–11.97 g/dL, lower than those in HV (14.33–15.23 g/dL). The mean serum EPO concentration was 6.64–14.33 mIU/mL

in CKD patients and 7.97–10.98 in HV. Consistent with results of previous reports [11], baseline serum EPO concentrations were not increased in CKD patients in comparison with those in HV despite of lower Hb concentration. Detailed patient demographics is presented in online supplementary Materials and Table S1 (for all online supplementary material, see www.karger.com/doi/10.1159/000492181).

Pharmacokinetics

Plasma concentration-time profiles of TP0463518 after a single oral administration are shown in Figure 1; summary of PK parameters are shown in online supplementary Materials and Table S2. Plasma exposure (C_{max} and $AUC_{0-\infty}$) of TP0463518 increased in a dose-dependent manner within each subject group (HV, ND, and HD patients). Fe_{0-24h} were 65.6–80.7% and 50.9–55.7% in HV and ND patients, whereas <1% of TP0463518 was excreted in HD patients. Mean $t_{1/2}$ values were 5.91–7.41, 12.6–12.8, and 25.8–27.6 h in HV, ND, and HD patients respectively. A dose of 11 mg TP0463518 was administered after a meal to each subject group, resulting in an increase in mean $AUC_{0-\infty}$ in ND and HD patients compared with that in HV (3.2- and 9.1-fold values respectively). As for the effect of food examined in 11-mg group of HV, the ratio of the geometric mean with 90% CI for C_{max} was 0.69 (0.52–0.93) in the fasting state and post-prandial were 526 ± 169 and 356 ± 93.8 ng/mL, respectively, indicating a 31% reduction due to ingestion of a meal.

Pharmacodynamics

As mentioned above, baseline values of serum EPO concentrations were similar across the groups, ranging from 6.64 to 14.33 mIU/mL.

Dose-related increases in serum EPO concentrations were observed in each subject group (Fig. 2). Serum EPO concentration began to increase 4 h after TP0463518 administration in all subject groups (HV, ND, and HD patients), and it reached a maximum level at 8 h in HV, 12 h in ND patients, and 24 h in HD patients. The elevation of serum EPO concentration was delayed due to the quick elevation of plasma TP0463518. ΔE_{max} of serum EPO concentration in the 11-mg groups of HV, ND patients, and HD patients were 24.37 ± 11.37 , 201.57 ± 130.34 , and $1,324.76 \pm 1,189.24$ mIU/mL ($n = 6, 10$, and 5) respectively.

The relationship between PK parameters (C_{max} and $AUC_{0-\infty}$) and PD parameter (ΔE_{max}) was analyzed using combined data of all subject groups (Fig. 3). A correlation was observed between the logarithmic conversion of ΔE_{max} and C_{max} ($R = 0.600$), but ND and HD patients showed higher serum EPO concentrations than HV at the same C_{max}

Fig. 1. Mean plasma concentration-time profiles of TP0463518 (mean $\pm$ SD) in HV (a), ND patients (b), and HD patients (c) for linear-scale and in the 11-mg group in each subject group for semi-logarithmic scale (d). Follow-up 1 in HD patients was treated as

72 h. TP0463518 was administered under fasting state in (a) and after meal in (b–d). HD, CKD patients undergoing hemodialysis; HV, healthy volunteers; ND, CKD patients not undergoing dialysis.

(Fig. 3a). $\Delta E_{\max}$ was more strongly correlated to $AUC_{0-\infty}$ than to $C_{\max}$, with correlation coefficients between logarithmic conversions of $\Delta E_{\max}$ and $AUC_{0-\infty}$ of 0.945, and no difference was observed between the groups (Fig. 3b).

Changes from baseline in the reticulocyte count after the administration of 11-mg TP0463518 at Follow-up 2 (1 week after administration) were $-0.183 \pm 0.817 \times 10^4/\mu\text{L}$, $2.31 \pm 1.49 \times 10^4/\mu\text{L}$, and $6.12 \pm 3.58 \times 10^4/\mu\text{L}$ in HV, ND patients, and HD patients respectively. Slight increases in the reticulocyte count were observed in ND and HD patient groups on Follow-up 2, whereas no such trend was observed in HV.

Safety

No serious AEs or drug-related AEs were reported; no subjects were discontinued in both studies. No clinically significant changes were observed in vital signs, electrocardiograms, and laboratory parameters in both studies.

EPO Glycosylation Pattern Analysis

Figure 4a shows EPO glycosylation patterns determined as PMI in placebo and 20-, and 36-mg groups of HV, with umbilical cord blood as a control for mainly liver-derived EPO. In HV, PMI values in 20- and 36-mg groups were higher than those in the placebo group and similar to those of umbilical cord blood. Point estimates with 95% CI for the difference in 20- and 36-mg groups versus the placebo group were 37.3% (29.0–45.7) and 42.2% (32.1–52.3) respectively.

Figure 4b shows PMI values in 6- and 11-mg groups of ND patients and the 11-mg group of HD patients. Peripheral blood and umbilical cord blood were used as controls for mainly kidney-derived and liver-derived EPO. In the ND participants, PMI values pre-dose were similar to that of peripheral blood but elevated to a level similar to the umbilical cord blood following TP0463518 administra-

Fig. 2. Changes in mean serum EPO concentration over time (mean $\pm$ SD) in HV (a), ND patients (b), and HD patients (c) for linear-scale and in the 11-mg groups in each subject group for semi-logarithmic scale (d). Follow-up 1 in HD patients was treated

as 72 h. EPO, erythropoietin; HD, CKD patients undergoing hemodialysis; HV, healthy volunteers; ND, CKD patients not undergoing dialysis.

Fig. 3. Relationship between PK parameters [C_{max} (a) and $AUC_{0-\infty}$ (b)] and PD parameter (ΔE_{max}) across the studies. $AUC_{0-\infty}$, area under the concentration-time curve extrapolated to infinity; C_{max} ,

the maximum concentration; E_{max} , the maximum serum EPO concentrations; HD, CKD patients undergoing hemodialysis; HV, healthy volunteers; ND, CKD patients not undergoing dialysis.

Fig. 4. The EPO glycosylation pattern determined by PMI (mean ± SD) in the HV (a) and CKD studies (b). HD, CKD patients undergoing hemodialysis; HV, healthy volunteers; ND, CKD patients not undergoing dialysis; PMI, percentage of migrated isoform.

tions in both groups. In HD patients, PMI values were similar to that of umbilical cord blood at pre-dose, and further increased after TP0463518 administration. Point estimates with 95% CI for the change from baseline in 6- and 11-mg groups of ND patients and in the 11-mg group of HD patients were 28.4% (19.0–37.9), 34.6% (27.0–42.1), and 26.5% (6.5–46.6) respectively.

Discussion

The aim of these studies was to characterize PK, PD, and safety profile of TP0463518 and to elucidate the origin of EPO induced by TP0463518 in HV and CKD patients.

As shown in online supplementary Materials and Table S2, >65% of the dose was excreted in urine as unchanged form of TP0463518 in HV, which suggests that the main elimination pathway of TP0463518 is renal. Also, ND and HD patients showed that elimination of TP0463518 was delayed and plasma exposure increased. Although only <1% was excreted in urine, TP0463518 was eliminated with a $t_{1/2}$ of approximately 27 h in HD patients. In addition, no metabolites were detected in the urine or plasma from HD patients (data not shown). These findings suggest that TP0463518 has multiple elimination pathways, apart from the kidney, such as biliary excretion. Because it was confirmed that TP0463518 is excreted even in HD

patients, it is possible that these patients could also benefit from the treatment.

A dose-dependent increase in serum EPO concentrations after oral administration of TP0463518 was observed in each subject group (Fig. 2). On comparison between subject groups administered the same dose, $\Delta E_{\max}$ of serum EPO concentration was the highest for HD patients, followed by ND patients and HV. In scatter plots shown in Figure 3, the correlation coefficient of log-transformed values between $\Delta E_{\max}$ and $AUC_{0-\infty}$ was higher than that between $\Delta E_{\max}$ and $C_{\max}$, which implies that the increase in EPO concentrations was more largely dependent on $AUC_{0-\infty}$ than on $C_{\max}$ in the subjects. Because the main excretion route for TP0463518 is renal, the functioning of this system may influence plasma concentration of TP0463518. TP0463518 demonstrates a good exposure-response relationship for all participants enrolled in the studies irrespective of their renal function. These data indicate that higher serum EPO concentrations in patients with reduced renal function are related to higher plasma exposure.

TP0463518 was well tolerated at single doses of up to 36 mg in HV and up to 11 mg in ND and HD patients. No major safety concerns were identified in these studies.

The glycosylation pattern of EPO depends on the producing cell and thus, can indicate its origin [12]. In the HV study, PMI values were elevated after TP0463518

administration from similar levels of peripheral blood, which contains mainly kidney-derived EPO, to similar levels of umbilical cord blood, which contains mainly liver-derived EPO (Fig. 4). In CKD study, PMI value in ND patients was similar to that in peripheral blood and lower than umbilical cord blood, which is different from the result in de Seigneux et al. [12]. Variation of PMI value may be large between samples and assays, but baseline PMI value greatly increased after TP0463518 administration in ND patients. Therefore, we think that most part of EPO induced by TP0463518 is produced by liver. In HD patients, baseline PMI values were higher than those in peripheral blood and similar to umbilical cord blood in consistent with results of a previous report [12], which suggests that some of EPO in HD patients originates in the liver to compensate for insufficient renal EPO production. After TP0463518 administration in HD patients, PMI values were even higher than umbilical cord values (84.4 ± 3.5 vs. $57.2 \pm 5.5\%$). Although liver is known to be the main site of EPO production in the fetus, kidneys also contribute up to 27% of the total EPO gene expression beyond 30 weeks of gestation [7]. Hence, the high PMI values in HD patients suggest that the fraction of liver-derived EPO in HD patients is higher than that in umbilical cord blood. These results imply that EPO secretion induced by TP0463518 is predominantly produced in the liver regardless of the renal function.

It is justifiable to be concerned that EPO production in kidney induced by HIF stimulation would be reduced or even lost in patients with end-stage renal disease. Therefore, TP0463518, which can induce EPO production mainly in the liver, and not in the kidneys, may allow sustained and stable EPO production in CKD patients.

High doses of TP0463518 in CKD patients tended to increase the number of reticulocytes, which was sufficient to increase Hb in other HIF-PHIs studies [13, 14]. The glycosylation pattern of EPO derived from the liver is different from that of EPO derived from the kidney. Glycosylation changes that may influence both EPO binding affinity to its receptor and its half-life and the efficiency of liver-derived EPO regarding erythropoiesis are currently unknown [12]. The increase in reticulocytes following TP0463518 administration suggests that the liver-specific EPO production may increase Hb and is a new strategy for the treatment of anemia [5].

Present studies have the following limitations. First, any potential increases in Hb could not be measured because of the short duration of these studies. Second, the

change in the glycosylation pattern of EPO is only an indirect method for identifying the predominant organ of EPO production, and it cannot evaluate the amount of EPO from each organ. Therefore we cannot exclude the possibility of EPO production from kidney or other organs. Further studies are recommended to confirm these findings and support the proposed hypothesis.

In summary, these studies provided detailed PK and PD profiles of TP0463518. The compound induces a dose-dependent increase in EPO production, primarily synthesized in the liver, and this is seen in HV and CKD patients. These initial results suggest that TP0463518 has the potential to be a new treatment option for anemia in CKD, which can be used regardless of renal functions. Long-term studies are needed for assessing Hb response and further safety properties of TP0463518.

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Ethics Statement

Both studies were conducted in accordance with the Declaration of Helsinki, standards of the Japanese Pharmaceutical Affairs Law and Good Clinical Practice. Study protocols were reviewed and approved by the institutional review boards of all participating institutions. All subjects provided written informed consent.

Disclosure Statement

All authors are employees of Taisho Pharmaceutical Co., Ltd.

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Author Contributions

M.F. and F.S. designed the studies; A.S., T.Sh., H.W., and T.N. conducted the clinical studies; H.W., Y.K., M.M., and H.Y. analyzed data and prepared the figures; N.T. and S.K. conducted experiments for determination of EPO glycosylation patterns; A.S. and T.Sh. drafted and revised the paper; T.Su. and J.M. were responsible for the total management of the studies. All authors approved of the final version of the manuscript.

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