

Pharmacokinetics and Safety of Defibrotide in Healthy Japanese Subjects

Clinical Pharmacology
in Drug Development
2016, 5(6) 548–551
© 2016, The Authors. Clinical
Pharmacology in Drug Development
Published by Wiley Periodicals, Inc. on
behalf of The American College of
Clinical Pharmacology
DOI: 10.1002/cpdd.262

**Kazuo Umemura¹, Takayuki Iwaki¹, Toshimi Kimura², Chitose Ogawa³,
Takahiro Fukuda⁴, Shuichi Taniguchi⁵, Keizo Horibe⁶, Hiroaki Goto⁷,
Kenichi Yoshimura⁸, Yasutaka Watanabe⁹, Chika Nitani³, and Atsushi Kikuta¹⁰**

Keywords

defibrotide, pharmacokinetics, drug safety, fibrinolysis, coagulation

Veno-occlusive disease (VOD) is a serious complication commonly occurring after stem cell transplantation (SCT). In VOD, sinusoidal endothelial cells and hepatocytes are damaged because of progressive venular occlusions, leading to hepatocellular necrosis.^{1–3} VOD severity ranges from mild to severe; severe VOD is defined by the presence of multiorgan failure and is associated with a greater than 85% 100-day mortality rate.^{4–6} VOD currently has no approved prophylactic or curative treatment in Japan.

Defibrotide (DF) is a polydisperse mixture of 90% single-stranded polydeoxyribonucleotides that has antithrombotic, antiischemic, and profibrinolytic functions.^{7–10} DF, which is given 4 times a day at a dose of 6.25 mg/kg, has been approved by the European Medicines Agency for the treatment of severe hepatic VOD in SCT therapy. It is an investigational drug that has been granted orphan drug status by the Food and Drug Administration.

To support the clinical development of DF for VOD treatment in Japan, we investigated the pharmacokinetics and evaluated the safety of DF in healthy Japanese subjects.

Methods

Subjects

The protocol was approved in advance by the Human Institutional Review Board of Hamamatsu University School of Medicine, Hamamatsu, Japan. Written informed consent was obtained from each subject before participation in this study.

Twenty healthy male Japanese subjects between 20 and 43 years old who weighted between 55.8 and 70.9 kg were enrolled in the study. Physical examination and standard biochemical, hematologic, and urinalysis

screening tests were conducted to assess the suitability of the test subjects. The subjects took no other medication during the week leading up to and for the duration of the study. The consumption of alcohol and caffeinated beverages was prohibited during the study.

Study Design

This study had a randomized, single-blind, placebo-controlled study design. The 20 subjects were randomly divided into 2 groups. From each group, 8 subjects

¹Department of Pharmacology, Hamamatsu University School of Medicine, Hamamatsu, Japan

²Department of Pharmacy, Tokyo Women's Medical University Hospital, Tokyo, Japan

³Department of Pediatric Oncology, National Cancer Center Hospital, Tokyo, Japan

⁴Department of Hematopoietic Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan

⁵Department of Hematology, Toranomon Hospital, Tokyo, Japan

⁶Clinical Research Center, National Hospital Organization Nagoya Medical Center, Nagoya, Japan

⁷Division of Hemato-Oncology/Regenerative Medicine, Kanagawa Children's Medical Center, Yokohama, Japan

⁸Innovative Clinical Research Center, Kanazawa University, Kanazawa, Japan

⁹Clinical Trial Support Center, National Cancer Center Hospital East, Tokyo, Japan

¹⁰Department of Pediatric Oncology, Fukushima Medical University, Fukushima, Japan

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Submitted for publication 8 November 2015; accepted 3 March 2016.

Corresponding Author:

Kazuo Umemura, MD, PhD, Department of Pharmacology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan (e-mail: umemura@hama-med.ac.jp)

received either a 3.0 or 6.25 mg/kg dose of DF, and 2 subjects received placebo. Because DF has yet to be administered to many Japanese patients, we first administered the 3.0 mg/kg dose, as a 2-hour infusion to verify dose safety. Once this dose was deemed safe, the second group received a 6.25 mg/kg dose, administered as a 2-hour infusion.

Tolerance

Subjective and objective symptoms as well as vital signs, including blood pressure, heart rate, and body temperature, were recorded before treatment and periodically for 24 hours after DF administration. Routine laboratory tests, including hematology, blood biochemistry, and urinalysis, were also performed. Twelve-lead electrocardiograms were recorded immediately before and 24 hours after DF administration.

Pharmacokinetic Analysis

Blood samples for pharmacokinetic analysis were collected in heparinized tubes prior to DF administration, 1 and 2 hours after the start of administration, and 5, 15, 30, and 60 minutes after administration completion. All plasma samples were stored below -80°C until assayed. Gentium S.p.A developed the analytical method for plasma DF quantification, and it was validated by Shin Nippon Biomedical Laboratories, Ltd. (Pharmacokinetics and Bioanalysis Center, Japan) for use in this study. Plasma samples (50 μL) were prepared by adding 50 mmol/L ammonium acetate (450 μL) and filtered through a 0.45- μm centrifuge filter (Centricut Mini, Kubota, Japan) by centrifuging at 5000g for 5 minutes. The percentage of drug recovered from the plasma was 110.7%, 85.8%, and 79.7% for 30, 140, and 225 $\mu\text{g}/\text{mL}$ DF doses, respectively. Concentrations of DF in the plasma were measured using high-performance liquid chromatography (Waters 2690 system) equipped with a C8 Column (3.5 μm , 2.1×100 mm, Waters X Bridge) at 50°C in a constant temperature bath and a UV detector (Waters 2487). DF was detected at a wavelength of 260 nm. The mobile phase was composed of 25 mmol/L ammonium acetate buffer (pH 9.0, mobile phase A), 50 mmol/L ammonium acetate buffer (pH 9.0)/acetonitrile/methanol (50/45/5 v/v/v, mobile phase B), 1% ammonia solution (mobile phase C), and acetonitrile (containing 0.1% formic acid)/ultra-pure water (9/1 v/v, mobile phase D). The following parameters were employed: flow rate of 0.4 to 0.5 mL/min and 20- μL injection volume. Accuracy was determined by calculating the relative error (RE %) based on the theoretical concentrations, calculated as follows: (measured concentration - theoretical concentration)/theoretical concentration $\times 100$ (the absolute value of 100 minus the average estimated concentration divided by the

theoretical concentration). The lower limit of quantification (LLQ) was 10 $\mu\text{g}/\text{mL}$, and the RE was within $\pm 15\%$, with the exception of the values of the LLQ. The coefficients of variation (CV%) within and between days in detecting from 10 to 300 $\mu\text{g}/\text{mL}$ were less than 3.1% and 6.9%, respectively.

Pharmacokinetic parameters, including time to maximum plasma concentration (T_{max}), plasma half-life ($t_{1/2}$), area under the plasma concentration-time curve (AUC), and total plasma clearance ($[\text{CL}]_{\text{tot}}$), were analyzed by standard noncompartmental methods using the computer program WinNonlin 5.2.1 (Pharsight Corporation). AUC_{0-3} was estimated by the linear trapezoidal rule, and $\text{AUC}_{0-\infty}$ was determined using $\text{AUC}_{0-3} + \text{AUC}_{3-\infty}$, which was calculated by dividing the concentration at 3 hours by the elimination rate constant value of the terminal slope.

Pharmacodynamic Analysis

Blood samples were obtained prior to treatment and 2 and 24 hours after the start of the 2-hour DF infusion to determine the fibrinolytic and coagulation activities. The parameters studied included prothrombin time, activated partial thromboplastin time, and levels of the following coagulation and fibrinolytic factors: fibrinogen, fibrinogen/fibrin degradation products, D-dimer, plasminogen activator inhibitor-1 antigen, protein C antigen, $\alpha 2$ -plasmin inhibitor, plasmin/ $\alpha 2$ -plasmin inhibitor complex, plasminogen activity, thrombin-antithrombin complex, tissue factor pathway inhibitor (TFPI), platelet factor 4, thromboxane- B_2 , and 6-keto-prostaglandin $\text{F}_{1\alpha}$.

Statistical Analysis

Data are presented as mean \pm standard deviation (SD). Analysis of variance (ANOVA) and Dunnett's test were used for comparison of the pharmacodynamics data. The statistical analysis was performed using SAS (SAS Institute) and SPSS (IBM). Results were deemed significant at $P < .05$.

Results

Safety and Tolerance

One subject exhibited mild disorders in liver function when administered 3.0 mg/kg DF. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values increased 7 days postadministration and returned to normal by the 17th postadministration day (AST, 26 IU/L prior to administration, 20 IU/L 24 hours after administration, 46 IU/L on day 7, 18 IU/L on day 17; ALT, 29, 21, 58, and 18 IU/L, respectively).

No other abnormalities in objective symptoms or in laboratory test results, including abnormalities in blood

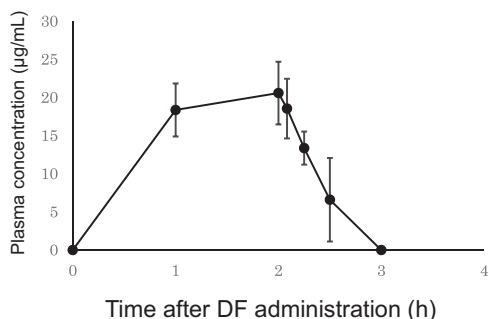


Figure 1. The defibrinolytic (DF) plasma concentration peaks immediately after the completion of a 2-hour infusion of 6.25 mg/kg DF. Blood plasma DF levels were determined using high-performance liquid chromatography. Data represent mean \pm standard deviation (SD); $n = 8$. Plasma concentrations from a 3.0 mg/kg DF were below the assay's limit of detection.

pressure, heart rate, electrocardiogram, body temperature, hematology, blood chemistry, and urinalysis, were attributable to DF.

Pharmacokinetics of DF

The changes in DF plasma concentrations after infusion with a 6.25 mg/kg DF dose are shown in Figure 1, and the associated pharmacokinetic parameters are shown in Table 1. When 3.0 mg/kg of DF was administered, plasma concentrations were below the limit of detection. On administration of 6.25 mg/kg DF, the mean values for the maximum observed plasma concentration C_{max} and $AUC_{0-\infty}$ were 20.59 ± 4.11 ng/mL and 42.32 ± 6.95 ng·h/mL, respectively. The mean values for $t_{1/2}$ and $(CL)_{tot}$ were 0.47 ± 0.10 hours and 9.629 ± 1.175 L/h, respectively.

Pharmacodynamics of DF

Compared with placebo, a 6.25 mg/kg dose of DF significantly ($P < .001$) increased the plasma concentration of TFPI 2 hours after the start of infusion (Figure 2). No other fibrinolytic or coagulation parameters were affected by DF administration.

Discussion

Throughout the investigation period, no abnormalities attributable to the test drug were observed. Objective symptoms, vital signs, and routine laboratory test re-

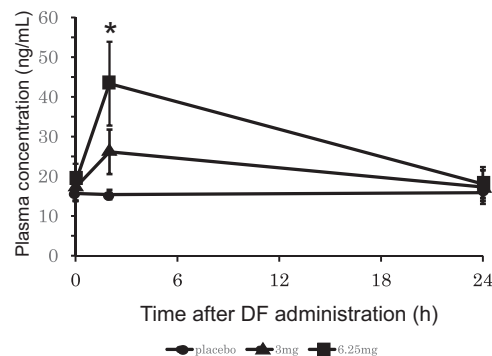


Figure 2. The human blood plasma tissue factor pathway inhibitor (TFPI) concentration peaked 2 hours after the start of the defibrinolytic (DF) treatment. Plasma TFPI levels were determined in DF-treated (infusion over 2 hours) healthy male Japanese subjects. Data represent mean \pm standard deviation (SD); $n = 4-8$ subjects per group. * $P < .05$ compared with placebo.

sults were not altered, suggesting that DF is well tolerated in healthy subjects.

The pharmacokinetic parameters observed in the present study were approximately the same as those obtained from the clinical trial on healthy subjects performed in the United States.¹¹ This suggests that there are no differences in the pharmacokinetic parameters of DF between Japanese and American subjects.

A previous study showed that DF reduces vascular permeability and inhibits the inflammation-induced expression of leukocyte adhesion molecules.¹² In addition, DF has been shown to have antithrombotic properties attributed to an increase in tissue plasminogen activator activity and a reduction in PAI-1 concentration.¹² Although in this study the plasma TFPI levels increased with administration of 6.25 mg/kg DF, the fibrinolytic and coagulation activities were not altered. These findings suggest that DF may not affect fibrinolytic and coagulation activity in healthy Japanese subjects.

Conclusions

From our observations, it can be concluded that DF has an acceptable pharmacokinetic profile and causes no serious adverse effects in healthy Japanese subjects.

Table 1. Pharmacokinetic Parameters of a 6.25 mg/kg Dose of Defibrinolytic in Healthy Japanese Subjects

	C_{max} (μ g/mL)	T_{max} (h)	kel (1/h)	$T_{1/2}$ (h)	AUC_{0-3} (μ g·h/mL)	$AUC_{0-\infty}$ (μ g·h/mL)	$(CL)_{tot}$ (L/h)	V_d (L)
n	8	8	8	8	8	8	8	8
Mean	20.59	2.00	1.55	0.47	37.09	42.32	9.27	7.31
SD	4.11	0.00	0.33	0.10	7.82	6.95	1.18	1.25

kel, elimination rate constant, V_d , volume of distribution.

Declaration of Conflicting Interests

Gentium provided the investigational drug and information deemed important to conduct the clinical trial. The article has been edited and proofread by a professional scientific editing company, Editage (www.editage.com).

Funding

This study was supported by Health and Labour Sciences Research Grants for Clinical Trial on Development of New Drugs and Medical Devices from the Ministry of Health, Labour and Welfare of Japan (15lk0201009h0004).

References

1. Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med*. 2006;354(17):1813–1826.
2. McDonald GB, Sharma P, Matthews DE, et al. Venocclusive disease of the liver after bone marrow transplantation: diagnosis, incidence, and predisposing factors. *Hepatology*. 1984;4(1):116–122.
3. Richardson P, Guinan E. The pathology, diagnosis, and treatment of hepatic veno-occlusive disease: current status and novel approaches. *Br J Haematol*. 1999;107(3):485–493.
4. McDonald GB, Hinds MS, Fisher LD, et al. Venocclusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. *Ann Intern Med*. 1993;118(4):255–267.
5. Coppel JA, Richardson PG, Soiffer R, et al. Hepatic veno-occlusive disease following stem cell transplantation: incidence, clinical course, and outcome. *Biol Blood Marrow Transplant*. 2010;16(2):157–168.
6. Carreras E, Bertz H, Arcese W, et al, and the European Group for Blood and Marrow Transplantation Chronic Leukemia Working Party. Incidence and outcome of hepatic veno-occlusive disease after blood or marrow transplantation: a prospective cohort study of the European Group for Blood and Marrow Transplantation. *Blood*. 1998;92(10):3599–3604.
7. Paul W, Gresele P, Momi S, et al. The effect of defibrotide on thromboembolism in the pulmonary vasculature of mice and rabbits and in the cerebral vasculature of rabbits. *Br J Pharmacol*. 1993;110(4):1565–1571.
8. Bianchi G, Barone D, Lanzarotti E, et al. Defibrotide, a single-stranded polydeoxyribonucleotide acting as an adenosine receptor agonist. *Eur J Pharmacol*. 1993;238(2-3):327–334.
9. Zhou Q, Chu X, Ruan C. Defibrotide stimulates expression of thrombomodulin in human endothelial cells. *Thromb Haemost*. 1994;71(4):507–510.
10. Falanga A, Vignoli A, Marchetti M, et al. Defibrotide reduces procoagulant activity and increases fibrinolytic properties of endothelial cells. *Leukemia*. 2003;17(8):1636–1642.
11. Tocchetti P, Ballabio M, Tudone E, et al. A phase 1, open-label study of defibrotide pharmacokinetics in severe/end-stage renal disease patients not on dialysis compared with healthy matching subjects. Presented at: 41st Annual Meeting of the European Society for Blood and Marrow Transplantation; March 22–25, 2015; Istanbul, Turkey.
12. Pescador R, Capuzzi L, Mantovani M, Fulgenzi A, Ferrero ME. Defibrotide: properties and clinical use of an old/new drug. *Vascul Pharmacol*. 2013;59(1-2):1–10.