Clinical Pharmacology in Drug Development
2015, 4(3) 237–241

DOI: 10.1002/cpdd.168

Assessment of Relative Bioavailability of Two Presentations of Moroctocog Alfa (AF-CC) in Subjects with Moderately Severe or Severe Hemophilia A

Frank Shafer[1], Robert J. Charnigo[1], Anna Plotka[1], James Baumann[1], Yali Liang[2], and Joan Korth-Bradley[1]

Abstract

An open-label, single-dose, randomized, two-period, cross-over study comparing the pharmacokinetics of factor VIII activity in plasma (FVIII:C) after administration of a new presentation of moroctocog alfa containing 3,000 IU in a dual-chamber syringe and the combined contents of approved 1,000 and 2,000 IU vials was conducted in 16 male subjects who had moderately severe or severe hemophilia A (FVIII:C $\leq$2 IU/dL). Blood samples were collected for 72 hours after administration of the dose. FVIII:C were assayed using a chromogenic substrate assay in a central laboratory. The FVIII:C pharmacokinetic parameters were calculated using noncompartmental analysis. The dual-chamber syringe would be bioequivalent to the combined contents of the vials if the 90% confidence limits of the ratio of the geometric mean values of AUC_{inf}, and C_{max} fell within the interval of 80–125%. The bioequivalence criteria were met. A total of seven treatment related adverse events were observed in a total of five subjects. All were mild and none was determined to be related to administration of study medication.

Keywords

hemophilia A, factor VIII, moroctocog alfa, pharmacokinetics

Recombinant technology has been used to develop synthetic molecules, which have similar pro-coagulant activity as endogenous coagulation factors missing in patients with hemophilia, and thus eliminating the potential for transmission of human blood-borne viruses during treatment. Moroctocog alfa, also referred to as B-domain deleted recombinant blood coagulation factor VIII (BDDrFVIII), differs from endogenous factor VIII by the lack of the B-domain. This product was available commercially as ReFacto. A revision to the manufacturing processes has allowed preparation of the drug without the use of albumin and is designated moroctocog alfa (AF-CC). It is commercially available as ReFacto AF or Xyntha. The two products, which are not available in the same market, differ by the method used to assign potency and consequently the amount of active ingredient in the dosage forms, but contain the same active pharmaceutical ingredient.

Development of products for the treatment of hemophilia must be consistent with regulatory guidances that include directions for both plasma-derived and recombinant products.[1,2] Per the guidance, significant changes in the manufacturing process require assessment of immunogenicity and pharmacokinetics to assure the similarity of active ingredient made by the new process to active ingredient made by the former process. Although regulatory guidances for non-hemophilia related medications allow sponsors to request waivers for medications administered by IV route of administration, because of the concern for the development of neutralizing antibodies, also known as inhibitors, such is not the case of products used to treat hemophilia.

Regardless of the replacement factor administered, pharmacokinetic behavior is assessed by measuring factor activity in plasma, rather than by assaying plasma samples for the molecule administered. Concentrations may be reported as IU/mL or percent (%), which is equal to IU/dL.

[1]Pfizer Inc, Collegeville, PA, USA
[2]Pfizer Inc, Groton, CT, USA

Submitted for publication 14 April 2014; accepted 2 September 2014

Corresponding Author:
Joan M. Korth-Bradley, Pfizer Inc., 500 Arcola Road, Collegeville, PA 19426, USA
(e-mail: joan.korth-bradley@pfizer.com)
ACCP Member: Joan Korth-Bradley (Fellow).
Clinical Trials.gov identifier: NCT 01579903.

Because of the use of FVIII:C to evaluate exposure following administration of hemophilia products, pharmacokinetic studies are not usually assessed in healthy volunteers. Results obtained in the presence of normal FVIII concentrations (100 IU/dL) would be difficult to extrapolate to individuals where endogenous FVIII concentrations are less than 1 or 2 IU/dL. Recommended sampling times are also dictated by regulatory guidances.[1,2] In addition to reporting conventional pharmacokinetic parameters that are available for other medications, studies of coagulation factors also report a parameter called recovery,[3] which is calculated as the ratio of change in FVIII:C after administration of a dose to the dose administered, is reported as IU/dL/IU/kg. Recovery is inversely related to volume of distribution.

The pharmacokinetics of FVIII:C after the administration of moroctocog alfa have been described in patients with hemophilia A after single doses[4–7] and have been shown to be bioequivalent to the pharmacokinetics observed after administration of purified plasma derived FVIII.[8] The FVIII:C mean ± SD parameters in 18 subjects with hemophilia after administration of 50 IU/kg moroctocog alfa were: peak concentration (C_{max}) 123 ± 19 IU/dL, area under the concentration time curve from time zero to infinity (AUC_{inf}) 22.2 ± 6.9 IU h/mL, half-life 14.8 ± 5.6 hours and recovery 2.43 ± 0.38 IU/dL/IU/kg.[4] Similar parameters were observed in another study of 18 subjects. The volume of distribution (Vss) and clearance (CL) were reported in the second study as 58.6 ± 13.7 mL/kg and 3.85 ± 1.36 mL/h/kg, respectively.[8]

The objective of the current study was to compare single-dose FVIII:C pharmacokinetics after administration of 3,000 IU moroctocog alfa (AF-CC) when administered by dual-chamber syringe to when administered as the combined contents of two vials of 1,000 and 2,000 IU in subjects with moderately severe or severe hemophilia A.

Subjects and Methods

Study Population

The study protocol was reviewed and approved by the following investigational sites' review boards: (Ethics Committee) MC "Comac Medical" Clinical Research Unit for Phase 1, BA/BE Studies, Sofia, Bulgaria (Egeszsegugyi Tudomanyos Tanacs), Klinikai Farmakologiai Etikai, Budapest, Hungary, and (NRES Committee) London Hampstead Northwick Park Hospital REC Center, Harrow, United Kingdom. A signed and dated, written, informed consent was required before any screen procedures were done. Sixteen male subjects with previously treated moderately severe or severe hemophilia A, with a mean ± SD age of 35 ± 13 years (range 18–61 years), weight of 76 ± 17 kg (range 51–104 kg) were enrolled in the study. Subjects had to have a negative test for FVIII inhibitor using the Bethesda Inhibitor Assay (<0.6 Bethesda units/mL), at the local laboratory at screening, and had to be able to comply with a 72-hour washout from FVIII-containing products prior to each administration of investigational product.

Study Product

The test product was a recently introduced dual-chamber syringe, which delivers 3,000 IU of moroctocog alfa (AF-CC) (ReFacto AF, Pfizer Inc., Wyeth Farma S.A., Madrid, Spain). The reference was a combined administration of two vials, which deliver 1,000 and 2,000 IU moroctocog alfa (AF-CC), respectively (ReFacto AF, Pfizer Inc.).

Study Methods

This study was conducted in compliance with the Declaration of Helsinki, with all International Conference on Harmonization (ICH) Good Clinical Practice (GCP) guidelines, as well as local regulatory requirements. The final protocols, amendments, and informed consent documentation were reviewed and approved by the independent ethics committees at each of the investigators' centers participating as noted above.

This study was an open-label, single-dose, randomized, two-period, crossover study and was consistent with guidances mentioned above.[1,2]

Subjects randomly received either test or reference study drug and after 7–28 days of washout, received the other presentation. Both presentations were administered by infusion over 2 minutes after a minimum of a 72-hour washout from products containing FVIII. Blood samples for determination of FVIII:C were collected before each infusion and then 0.25, 0.5, 1, 3, 6, 9, 12, 24, 48, and 72 hours after the start of the infusion. The actual times of sample collection were collected and used in the pharmacokinetic parameter calculations. Plasma was harvested as follows: blood samples were centrifuged, within 1 hour of collection, at a minimum of 2,000*g* for 10–20 minutes at 20–25°C until cells and plasma were well separated. The plasma was then removed and stored in screw-top polypropylene tubes and frozen immediately at −70°C.

Undiluted plasma samples (40 μL) were analyzed for FVIII:C using a validated chromogenic substrate assay (Chromogenix Coamatic Factor VIII Kit, DiaPharma Group, West Chester, OH, USA)[9] at a central laboratory (Covance Laboratories, Chantilly, VA, USA). The assay measured factor Xa generation, which was quantified by the amount of a chromogenic analyte, S-2222, generated and was referenced to the normal plasma standard (Dade Standard Human Plasma) which was calibrated against the WHO 4th International Standard for Blood Coagulation FVIII and von Willibrand factor in plasma. The

specific limits of quantitation depend upon the actual Dade standard used. The matrix used was Factor VIII-depleted human plasma containing sodium citrate (George King Bio-Medical, Overland Park, KS, USA). Calibration standards were freshly prepared on the day of the assay. During the performance of the samples from this study, the lower limit of quantitation was 0.007 IU/mL and the upper limit of quantitation was 0.900 IU/mL. Optical density response was quantified using a SpectraMax microplate reader and Softmax Pro 5.0.1 data acquisition and analysis software (Molecular Devices LLC, Sunnyvale, CA, USA). The maximum time from collection to analysis was 185 days, which was within the range of documented storage stability of 18 months.

Pharmacokinetic parameters were calculated for each subject using the plasma FVIII activity and the actual sample collection times. In clinical care settings, FVIII:C activity is conventionally reported in units of IU/dL. Although no conversion of FVIII:C data was made for calculation of most pharmacokinetic parameters, FVIII:$C_{0.5h}$, FVIII:C_{pre}, and graphical presentation of FVIII:C are reported as IU/dL. Conventional noncompartmental analysis parameters were calculated. Recovery was calculated using the FVIII:C reported at 0.5 hour after the start of the infusion and the weight observed on the day of study drug administration, using the equation:

$$\text{Recovery} = (\text{FVIII} : C_{0.5\,\text{h}} - \text{FVIII} : C_{\text{pre}})/(3,000\,\text{IU}/\text{weight})$$

FVIII:C measurements were summarized with mean and standard error for each formulation at each nominal time. Pharmacokinetic parameters were summarized for each formulation.

Natural log transformed AUC_{inf}, area under the FVIII:C versus time from time zero to the last reported FVIII:C (AUC_{last}) and C_{max} were analyzed using a mixed effect model with sequence, period, and treatment as fixed effects and subject within sequence as a random effect. Estimates of the adjusted mean differences (test–reference) and corresponding 90% confidence interval (CI) were obtained from the model. The adjusted mean differences and 90% CI for the differences were exponentiated to provide estimates of the ratio of adjusted geometric means (test/reference) and 90% CIs for the ratios.

Safety assessments, including testing for development of inhibitor to FVIII, were performed regularly throughout the study from screening until collection of the final specimen.

Results

All 16 subjects enrolled in the study received both formulations and completed all study procedures. All tests for inhibitors were negative. Five subjects reported a total of seven adverse events, but none were assessed to be treatment-related and all were mild in severity.

The mean ± standard error FVIII:C versus time profiles are shown in Figure 1. To link the observations to more familiar clinical terms, FVIII:C and C_{max} results are reported here with unit of IU/dL. Although all 16 subjects completed the study, a robust estimate of half-life (and consequently AUC_{inf}, clearance (CL), volume of distribution at steady state (Vss), and volume of distribution by area (V_{area})) could not be made in two subjects (one from dual-chamber group and the other from combined vials group) due to the limited number of samples observed during the terminal elimination phase. The pharmacokinetic parameters are shown in Table 1 and the bioequivalence assessment is shown in Table 2.

The relative bioavailability of moroctocog alfa (AF-CC) administered using the 3,000 IU dual-chamber syringe compared to the combined 1,000 and 2,000 IU vials was 91.4% (90% CI: 85.9–97.2%) and 95.4% (90% CI: 88.5%, 103%) based on AUC_{inf} and C_{max}, respectively. The CIs were within the acceptance range for bioequivalence.[10]

Discussion and Conclusions

The pharmacokinetic parameters observed in this study were consistent with what has been reported by others.[4,5,6,7,8] Recovery, which was assessed using the FVIII:$C_{0.5h}$ may have been underestimated compared to other investigations where C_{max} was used instead.

No new safety risks or concerns for moroctocog alfa (AF-CC) were identified.

The active drug product in the dual-chamber syringe is the same molecule as is found in the vials and thus would be expected to be bioequivalent as was shown and could be used interchangeably.

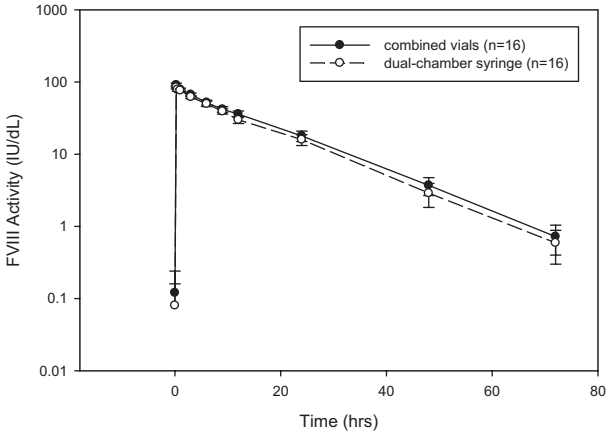

Figure 1. Mean ± SE FVIII activity versus time after 3,000 IU in subjects with moderate or severe hemophilia A.

Table 1. Descriptive Summary of Plasma Factor VIII Activity Pharmacokinetic Parameter Values Following Single IV Doses of Moroctocog Alfa (AF-CC)

	Parameter summary statistics[a] by treatment	
Parameter (units)	3,000 IU (dual-chamber)	3,000 IU (1,000 + 2,000)
N, n	16, 15	16, 15
AUC_{inf} (IU h/mL)	11.5 (±5.4)	12.8 (±5.7)
AUC_{last} (IU h/mL)	10.5 (±5.2)	11.9 (±5.6)
C_{30} (IU/dL)	78.47 (±16.99)	81.36 (±14.98)
C_{max} (IU/dL)	85.59 (±13.82)	90.99 (±21.69)
T_{max} (h)	0.25 (0.25–6)	0.25 (0.25–1.03)
$t_{½}$ (h)	9.52 (±3.02)	10.29 (±2.95)
CL (mL/h)	328 (±156)	288 (±151)
Recovery (IU/dL/IU/kg)	1.96 (±0.43)	2.02 (±0.34)
V_{ss} (mL)	4,014 (±656)	3,736 (±712)
V_{area} (mL)	3,922 (±576)	3,756 (±810)

IU, international unit; N, number of subjects in the treatment group; n, number of subjects with reportable AUC_{inf}, $t_{½}$, Vss, V_{area} and CL; SD, standard deviation.
[a]Arithmetic mean (±SD) for all except: median (range) for T_{max}.

Table 2. Statistical Summary of Treatment Comparisons for Plasma Factor VIII Activity Parameters Following Single IV Doses of Moroctocog Alfa (AF-CC)

	Adjusted geometric means			
Parameter (units)	3,000 IU (dual-chamber) (test)	3,000 IU (1,000 + 2,000) (reference)	Ratio (test/reference) of adjusted means[a]	90% CI for ratio
AUC_{inf} (IU h/mL)	10.34	11.32	91.4	85.9, 97.2
AUC_{last} (IU h/mL)	9.418	10.67	88.3	83.8, 93.0
C_{max} (IU/dL)	84.67	88.77	95.4	88.5, 103

IU, international unit; CI, confidence interval.
[a]The ratios (and 90% CIs) are expressed as percentages.

Acknowledgments

This study was sponsored by Pfizer Inc. The authors wish to acknowledge and thank the investigators Dr. Pratima Chowdary, Dr. Judit Kapocsi, and Professor Toshko Lissichkov and their patients who participated in the trial.

Declaration of Conflicting Interests

All of the authors are employees and stock holders of Pfizer Inc.

Funding

This study was funded by Pfizer Inc., the manufacturer of ReFacto AF, the study drug evaluated in the study.

References

1. EMA Committee for Medicinal Products for Human Use (CHMP). Guideline on the clinical investigation of recombinant and human plasma-derived factor VIII products. 2011. (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109692.pdf) accessed December 15, 2013.
2. Morfini M. Pharmacokinetic studies: international guidelines for the conduct and interpretation of such studies. *Haemophilia.* 2006;12(Suppl 4):6–11.
3. Bjorkman S, Folkesson A, Berntorp E. In vivo recovery of factor VIII and factor IX: intra- and interindividual variance in a clinical setting. *Haemophilia.* 2007;13:2–8.
4. Kessler CM, Gill JC, White GC, et al. B-domain deleted recombinant factor VIII preparations are bioequivalent to a monoclonal antibody purified plasma-derived factor VIII concentrate: a randomized three-way study. *Haemophilia.* 2005;11:84–91.
5. Lambert T, Guerois C, Gay V, et al. Factor VIII recovery after a single infusion of recalibrated ReFacto in 14 severe haemophilia A patients. *Haemophilia.* 2007;13:357–360.
6. Fijnvandraat K, Berntorp E, ten Cate JW, et al. Recombinant, B-domain deleted factor VIII (r-VIII SQ):

pharmacokinetics and initial safety aspects in hemophilia A patients. *Thrombosis Haemostasis.* 1997;77:298–302.
7. Rea C, Dunkerley A, Sorensen B, Rangarajan S. Pharmacokinetics, coagulation factor consumption and clinical efficacy in patients being switched from full-length FVIII treatment to B-domain-deleted r-FVIII and back to full-length FVIII. *Haemophilia.* 2009;15:1237–1242.
8. Di Paola J, Smith MP, Klamroth R, et al. ReFacto and Advate: a single-dose, randomized, two-period crossover pharmacokinetics study in subjects with haemophilia A. *Haemophilia.* 2007;13:124–130.
9. Rosen S, Casoni MC. Chromogenic determination of factor VIII activity in plasma and factor VIII concentrates. Factor VIII monograph. Available at: www.chromogenix.com. Accessed June 2014.
10. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for Industry. Statistical approaches to establishing bioequivalence; 2001.