Pharmacokinetics of recombinant factor XIII at steady state in patients with congenital factor XIII A-subunit deficiency

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Summary. Background: The use of monthly recombinant factor XIII (rFXIII) recently demonstrated favorable safety and efficacy for congenital FXIII A-subunit deficiency patients aged ≥ 6 years (mentor™1 trial), although the pharmacokinetics (PK) were not fully evaluated. Objectives: To comprehensively evaluate the steady-state pharmacokinetics (PK) were not fully evaluated. Patients/methods: To comprehensively evaluate the PK of rFXIII in patients aged ≥ 6 years with congenital FXIII A-subunit deficiency. Patients/methods: mentor™2 is an ongoing, multinational safety and efficacy trial in which patients are receiving monthly rFXIII (35 IU kg⁻¹) for ≥ 52 weeks. For this 28-day PK analysis, blood samples were collected immediately predosing, and 1 h, 2 h, 3, 7, 14, 21, and 28 days postdosing. FXIII activity was measured and PK parameters were calculated using non-compartmental analysis, without prior baseline adjustment. Information regarding adverse events and bleeding was collected at each visit. Antibody assessments were performed predosing and at day 28. Results: PK analysis in 23 patients revealed first-order elimination of rFXIII with a geometric mean half-life of 13.6 days. Mean FXIII activity was > 0.1 IU mL⁻¹ throughout the 28-day period, with a geometric mean peak activity of 0.87 IU mL⁻¹ and trough of 0.16 IU mL⁻¹. The geometric mean clearance was 0.15 mL h⁻¹ kg⁻¹. No bleeding episodes occurred during the PK session, and no anti-rFXIII antibodies were detected. Peak and trough FXIII activities were constant over time, compared with previous activities (≥ 10 rFXIII doses) in the same patients.

Conclusions: Clearance of rFXIII is unaffected over time, and monthly prophylaxis with 35 IU kg⁻¹ rFXIII provides FXIII activity > 0.1 IU mL⁻¹ throughout the dosing interval in patients with congenital FXIII A-subunit deficiency.

Keywords: factor XIII; factor XIII deficiency; fibrinolysis; pharmacokinetics; recombinant factor XIII-A2.

Introduction

Circulating heterotetrameric factor XIII (FXIII) becomes activated by thrombin and calcium into a transglutaminase that cross-links α2-antiplasmin to fibrin, stabilizing fibrinolytically resistant fibrin clots [1]. The heterotetramer consists of two A-subunits (FXIII-A) and two B-subunits (FXIII-B). FXIII-A is predominantly synthesized in hematopoietic cells and circulates in plasma bound to FXIII-B, which protects it from spontaneous activation and clearance. FXIII-B is synthesized predominantly by hepatocytes and circulates in molar excess to FXIII-A with ~ 50% of FXIII-B being free, uncomplexed protein [2]. Dimeric FXIII-A is also found in monocytes, histiocytes, and platelet cytoplasm [2].

Severe FXIII deficiency is a rare, autosomal recessive bleeding disorder, affecting ~ 1 in 2 million persons worldwide, with higher prevalence in cultures where consanguineous marriage is common [2]. Of the estimated 1126 worldwide cases, the vast majority are FXIII-A deficient [2,3]. FXIII-B deficiency has been described in < 20 families. Approximately 80% of patients present shortly after birth with umbilical stump bleeding. Without appropriate diagnosis and institution of prophylactic therapy, up to 30% of patients sustain intracranial hemorrhage [2]. Other manifestations such as impaired wound healing, bruising, hematomas, and oral mucosa bleeding, as well
as menorrhagia and recurrent pregnancy loss, are common.

Prophylactic replacement of FXIII, which significantly reduces bleeding events, has thus become the standard of care for severe deficiency [4-6]. Historically, this has been accomplished with blood products or plasma-derived FXIII (pdFXIII) [7]. Recombinant factor products represent an advancement over plasma-derived products, and completely understanding their pharmacokinetic (PK) properties is essential to their clinical development [7,8]. Recombinant FXIII-A (rFXIII, NovoThirteen™) is manufactured by Novo Nordisk A/S (Copenhagen, Denmark) through transgene expression in Saccharomyces cerevisiae [9]. In a phase 3a study (mentor™1), rFXIII was demonstrated as a safe and effective therapy for congenital FXIII-A deficiency in subjects aged ≥6 years [9]. Complete PK results for younger children (<6 years) were recently reported (mentor™4) [10]. In mentor™1, PK was estimated from only three time points [9], mentor™2 (Safety of Monthly Recombinant Factor XIII Replacement Therapy in Subjects With Congenital Factor XIII Deficiency: An Extension to Trial F13CD-1725) is an ongoing mentor™1 extension trial assessment the safety and efficacy of long-term rFXIII prophylaxis. The purpose of the present investigation was to evaluate the steady-state PK of rFXIII in mentor™2, based on a multi-time-point sample profile.

Materials and methods

Design

All 55 congenital FXIII-A deficiency patients participating in mentor™2 were asked to contribute to this steady-state PK assessment during monthly (28 ± 2 days) prophylactic rFXIII (35 IU kg⁻¹) therapy. PK samples were collected immediately before a scheduled dose (trough) followed by additional samples at 1 and 2 h postdosing as well as 3, 7, 14, 21, and 28 (± 2) days postdosing. Information regarding adverse events and bleeding episodes were collected at each visit.

The study was performed in accordance with the Declaration of Helsinki and the International Conference on Harmonization, and Good Clinical Practice. Each participant provided informed consent. The study was registered at ClinicalTrials.gov (NCT00978380).

Assays

FXIII activity The Berichrom® FXIII activity assay (Siemens Healthcare Diagnostics, Munich, Germany), which detects the activity of both recombinant and endogenous FXIII-A, regardless of whether the A-subunits are in complex with FXIII-B, was used as the primary PK variable [9]. The assay buffer was modified by adding bovine serum albumin and K₂-EDTA to the isotonic saline. The regular kit calibrator (normal plasma) was changed for rFXIII reference material, serially diluted in the assay buffer to make up a calibration curve, to achieve a lower limit of quantification of 0.1 IU mL⁻¹ with intra-assay and intralaboratory precision of 4.3% and 10.4%, respectively [9]. Otherwise, the assay was performed according to the manufacturer’s instructions, without blank subtraction.

Immunogenicity An anti-rFXIII antibody ELISA and an in vitro neutralizing antibody assay were used for immune response screening, confirmation, and characterization of anti-rFXIII antibodies [9].

Statistics

PK parameters were calculated using non-compartmental methods, without prior baseline adjustment. Data are presented as individual values, geometric means (g), and coefficient of variation (CV). PK parameters included area under the activity-time curve (AUC₀⁻₂₈ days) from day 0 (predosing) through day 28 (immediately before the next dose), measured peak plasma activity (Cₘₐₓ), measured activity before the next dose (Cₜᵣᵣᵩᵩ), half-life (t½), clearance (CL), and volume of distribution at steady state (Vₛₛ). AUC₀⁻₂₈ days was computed using the linear trapezoid rule, and t½ was calculated as ln(2)/λᵢ, where λᵢ was calculated as the slope of the log transformed FXIII activities versus time in the terminal phase. Calculations were based on the available data without imputation of missing values, except that AUC₀⁻₂₈ days was extrapolated if the day 28 sample was missing; t½ was calculated based on the day 3–28 samples, under the condition that at least two of four samples were available. In order to calculate AUC₀⁻₂₈ days, at least two of three samples from days 14–28 were required.

Results and discussion

Twenty-three (42%) of the eligible patients agreed to participate. Age, body mass index, gender, and race of the PK subcohort were comparable to those of the overall mentor™2 study population (Table 1). Each patient had previously received ≥10 doses (mean 34; range 10–50) of rFXIII. Eighty-six percent (139/161) of the expected FXIII activity measurements were evaluable. Hemolysis affected 55% (12/22) of the non-evaluable samples, and the remainder (10/22) were not collected due to cancelled appointments.

The cohort gxFXIII activity was ≥0.1 IU mL⁻¹ at each time point throughout the study period (Fig. 1). Similarly, with only two exceptions, all individual PK profiles were ≥0.1 IU mL⁻¹. One individual had an activity < 0.1 IU mL⁻¹ on day 14 with subsequent levels > 0.1 IU mL⁻¹. Another individual had activity
> 0.1 IU mL\(^{-1}\) on day 0 but < 0.1 IU mL\(^{-1}\) on day 28. The latter patient’s history included trough activities > 0.1 IU mL\(^{-1}\) for 98% (48/49) of previous doses.

The gxFXIII activity profile was similar to the estimated PK activities derived from only three time points in the mentor\(^{10}\) trial (data not shown) [9,10]. Peak plasma concentration (gxF\(_{\text{max}}\)) of 0.87 IU mL\(^{-1}\) (SD 0.2 IU mL\(^{-1}\)) was achieved with the 35 IU kg\(^{-1}\) dose decreasing to a day 28 trough (gxF\(_{\text{trough}}\)) of 0.16 IU mL\(^{-1}\) (SD 0.06 IU mL\(^{-1}\)), Table 2. Reassuringly, the gxF\(_{\text{trough}}\) was similar to the gxF predose activity (0.19 IU mL\(^{-1}\); \(P = 0.23\)). Thus, there was no significant change in FXIII activity with cumulative dosing. Estimated FXIII gxF incremental recovery [(peak FXIII – trough FXIII)/35 IU kg\(^{-1}\)], based on individual activity levels, was 0.020 IU mL\(^{-1}\) per IU kg\(^{-1}\) (CV 0.25) of administered rFXIII. The AUC\(_{0-28}\) days was 236 IU*h mL\(^{-1}\). The gxF\(_{t_{1/2}}\) was 13.6 days, and the gxF\(V_{\text{ss}}\) (69.9 mL kg\(^{-1}\)) was similar to estimated total blood volume, indicating that the majority of study drug resides within the intravascular space.

No safety issues or anti-rFXIII antibodies were detected during the study period. One patient with a history of transient, non-neutralizing anti-FXIII antibodies participated in this evaluation and exhibited PK similar to that of the remainder of the cohort (Fig. 1). No treatment requiring bleeding episodes occurred during the 28 \pm 2-day study.

### Table 1 Demographic characteristics of pharmacokinetics (PK) study participants

<table>
<thead>
<tr>
<th></th>
<th>PK population</th>
<th>Non-PK population</th>
<th>All</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n (%)</td>
<td>23 (42)</td>
<td>32 (58)</td>
<td>55 (100)</td>
<td>–</td>
</tr>
<tr>
<td>Age, yrs</td>
<td>30.7 (15.1)</td>
<td>29.2 (17.8)</td>
<td>29.8 (16.6)</td>
<td>0.73*</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>28 (7–58)</td>
<td>24 (8–77)</td>
<td>25 (7–77)</td>
<td>–</td>
</tr>
<tr>
<td>Median (range)</td>
<td>18 (78.3)</td>
<td>17 (53.1)</td>
<td>35 (63.6)</td>
<td>0.06**</td>
</tr>
<tr>
<td>White race, n (%)</td>
<td>17 (73.9)</td>
<td>17 (53.1)</td>
<td>34 (61.8)</td>
<td>0.12**</td>
</tr>
<tr>
<td>Treatment-requiring bleeds before enrollment in mentor(^{10})</td>
<td>1 (1)</td>
<td>2 (3)</td>
<td>3 (4)</td>
<td>0.76**</td>
</tr>
</tbody>
</table>

*Unpaired t-test of continuous baseline variables, comparing PK subjects with non-PK subjects. **The \(\chi^2\) test of categorical baseline variables, comparing PK subjects with non-PK subjects.
Recombinant factor XIII (rFXIII) pharmacokinetic (PK) parameters derived from FXIII-deficient patients participating in the PK study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Geometric mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>IU mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>(n = 23*)</td>
<td>0.89 (0.20)</td>
<td>0.86</td>
<td>0.87</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.57–1.24</td>
</tr>
<tr>
<td>C&lt;sub&gt;trough&lt;/sub&gt; (day 28)</td>
<td>IU mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>(n = 21*)</td>
<td>0.17 (0.06)</td>
<td>0.15</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05–0.32</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0–28&lt;/sub&gt;, IU*h mL&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>(n = 19*)</td>
<td></td>
<td>240.4 (49.0)</td>
<td>234.0</td>
<td>236.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>168.6–355.9</td>
</tr>
<tr>
<td>t&lt;sub&gt;1/2&lt;/sub&gt;, days</td>
<td>(n = 20*)</td>
<td></td>
<td>13.9 (3.5)</td>
<td>13.2</td>
<td>13.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10.1–24.6</td>
</tr>
<tr>
<td>Recovery, IU mL&lt;sup&gt;-1&lt;/sup&gt; per IU kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>(n = 20*)</td>
<td></td>
<td>0.02 (0.00)</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001–0.003</td>
</tr>
<tr>
<td>CL, mL h&lt;sup&gt;-1&lt;/sup&gt; kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>(n = 19*)</td>
<td></td>
<td>0.15 (0.03)</td>
<td>0.15</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.10–0.21</td>
</tr>
<tr>
<td>V&lt;sub&gt;ss&lt;/sub&gt;, mL kg&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>(n = 19*)</td>
<td></td>
<td>73.2 (24.8)</td>
<td>65.9</td>
<td>69.9</td>
</tr>
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<td></td>
<td></td>
<td></td>
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<td>44.0–150.3</td>
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</table>

C<sub>max</sub>, peak measured plasma activity concentration; C<sub>trough</sub> measured activity before the next dose was taken as the trough; AUC<sub>0–28</sub>, area under the activity-time curve; t<sub>1/2</sub>, half-life; CL, clearance; V<sub>ss</sub>, volume of distribution at steady state. *n, participants with complete data for the variable.

During steady-state prophylactic treatment, rFXIII has predictable trough activity ≥ 0.1 IU mL<sup>-1</sup> for the majority of patients. There is substantial variability in peak activity (SD 0.2 IU mL<sup>-1</sup>), with less variability in trough activity (SD 0.06 IU mL<sup>-1</sup>). rFXIII clearance is monoeponential, following first-order elimination with a t<sub>1/2</sub> of 13.6 days. In comparison, the t<sub>1/2</sub> of pdFXIII has been reported as 5–14 days [5,11–14]. Thus, rFXIII PK resembles that of the native protein. rFXIII is not expected to have efficacy in the setting of FXIII-B deficiency, however, because rFXIII elimination would be substantially faster, due to inadequate endogenous FXIII-B to protect it from degradation.

The reasons for the variability in peak (C<sub>max</sub>) activity are not clear. There was no correlation between peak activity and body mass index or known FXIII-A repositories: basal FXIII-B concentration, platelet count, or fibrinogen level [1]. To confirm their diagnoses, on enrollment in a mentor™ study, all subjects underwent full-length gene sequencing for both FXIII-A (F13A1) and FXIII-B genes (F13B); known FXIII-B deficiency mutations were not present in these patients. Thus, it is unlikely that subtle, B-subunit qualitative defects account for this finding. Significant variability in pdFXIII prophylactic dose requirement has been observed and may be explained by heterogeneity of intracellular FXIII expression or activity modifying polymorphisms, which may also be applicable for rFXIII [13]. Higher levels are required for pregnancy maintenance and surgical recovery than for routine prophylaxis, but published regimens are largely anecdotal [13]. In these situations, adequate therapy may require individual PK monitoring, as recommended for perioperative hemophilia care [15–17].

While a prolonged t<sub>1/2</sub> therapy is advantageous for decreasing infusion frequency, PK profiling can become onerous and, as reflected by the enrolment rate, significantly impacted this study. For example, in this study, 58.2% (32/55) of the patients who were invited to participate in the PK evaluation declined, citing that the number of visits were prohibitive to their daily requirements (i.e. work and/or school). These issues should be carefully considered in the design of future PK studies of prolonged t<sub>1/2</sub> hemostatic agents. Encouragingly, estimated PK based on only three time points (mentor™) were reflective of this more intense analysis [9,10].

Because the first international standard for FXIII was not established until 2004, caution must be exercised when comparing these PK data to existing literature [18]. Many published observational cohort studies and clinical trials conducted with pdFXIII and even the earliest reports with rFXIII used arbitrary units, rather than International Units (IU) [4,5,13,19–21]. Compounding this issue, available FXIII activity assays are either quasi-quantitative or have poor sensitivity, especially as the activity approaches zero (< 0.05 arbitrary unit mL<sup>-1</sup>) [22–25]. An important limitation of the Berichrom<sup>®</sup> FXIII activity assay used in this study is a lack of blank subtraction which may result in an overestimation of FXIII activity, especially at the lower end of detection, due to FXIII-independent NADH-consuming and ammonia-producing reactions [23,26–29]. Thus, the Berichrom<sup>®</sup> FXIII activity assay and technique used for this study has poor intra-assay precision at activities < 0.1 IU mL<sup>-1</sup>. Therefore, the trough values reported in this study should be considered in this context.

Prophylactic therapy to prevent spontaneous bleeding in congenital FXIII deficiency is the recommended standard of care [4–6,9]. This study demonstrates that a prophylactic regimen with 35 IU kg<sup>-1</sup> of rFXIII achieves steady-state mean FXIII activity levels ≥ 0.1 IU mL<sup>-1</sup> throughout a 28-day dose window for the majority of patients with FXIII-A deficiency.

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Addendum

M. Lundblad and R. Tehranchi are responsible for study concept and design and writing the protocol; B. Kerlin, B. Brand, A. Inbal, S. Halimeh, and D. Nugent are responsible for data acquisition; B. Kerlin, B. Brand, D. Nugent, M. Lundblad, and R. Tehranchi are responsible for analysis and interpretation of data; B. Kerlin is responsible for manuscript drafting; and B. Kerlin, B. Brand, A. Inbal, S. Halimeh, D. Nugent, M. Lundblad, and R. Tehranchi are responsible for critical revision and final draft of manuscript.

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Disclosure of Conflicts of Interest

B. Kerlin has received research support and honoraria for consulting from Bayer Healthcare US and Novo Nordisk A/S. B. Brand has received research support from Novo Nordisk A/S. A. Inbal has received research support and reimbursement for attending symposia/congresses from Novo Nordisk A/S. S. Halimeh has received research support from Novo Nordisk A/S and honoraria for speaking at events sponsored by Bayer, Baxter, CSL Behring, Grifols, Novo Nordisk, and Pfizer. M. Lundblad and R. Tehranchi are employees of Novo Nordisk A/S and hold Novo Nordisk stock options.

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