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ORIGINAL ARTICLE

Pharmacokinetics, clot strength and safety of a new fibrinogen concentrate: randomized comparison with active control in congenital fibrinogen deficiency

C. ROSS,* S. RANGARAJAN,† M. KARIMI,‡ G. TOOGEH,§ S. APTE,¶ T. LISSITCHKOV,** S. ACHARYA,†† M. J. MANCO-JOHNSON,‡‡ A. SRIVASTAVA,§§ B. BRAND,¶¶ B. A. SCHWARTZ,*** S. KNAUB††† and F. PEYVANDI‡‡‡

*Department of Hematology, St John's Medical College and Hospital, Bangalore, India; †Centre For Haemostasis and Thrombosis, St Thomas' Hospital, London, UK; ‡Hematology Research Center, Nemazee Hospital, Shiraz University of Medical Sciences, Shiraz; §Thrombosis Hemostasis Research Center, Tehran University of Medical Sciences, Tehran, Iran; ¶Sahyadri Speciality Hospital, Pune, Maharashtra, India; **Department of Hemorrhagic Diathesis and Anemia, Specialized Hospital for Active Treatment (SHAT) 'Joan Pavel', Sofia, Bulgaria; ††Cohen Children's Medical Center of New York, Northwell Health, New Hyde Park, NY, USA; ‡‡Hemophilia and Thrombosis Center, University of Colorado Anschutz Medical Campus, Aurora, CO, USA; §§Department of Haematology, Christian Medical College, Vellore, India; ¶¶Department of Haematology, University Hospital Zurich, Zurich, Switzerland; ***Clinical Research and Development, Octapharma, Hoboken, NJ, USA; †††Research and Development Department, Octapharma, Lachen, Switzerland; and ‡‡‡Angelo Bianchi Bonomi Hemophilia and Thrombosis Centre, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico and Luigi Villa Foundation, Department of Pathophysiology and Transplantation, Università degli Studi di Milano, Milan, Italy

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Essentials

- Congenital afibrinogenemia causes a potentially life-threatening bleeding and clotting tendency.
- Two human fibrinogen concentrates (HFCs) were compared in a randomized pharmacokinetic study.
- Bioequivalence was not shown for AUC_{norm}, which was significantly larger for the new HFC.
- Increases in clot strength were comparable, and no thromboses or deaths occurred in the study.

Summary. *Background:* Human fibrinogen concentrate (HFC) corrects fibrinogen deficiency in congenital a-/hypofibrinogenemia. *Objectives:* To assess pharmacokinetics (PK), effects on thromboelastometry maximum clot firmness (MCF), and safety of a new double virus-inactivated/eliminated, highly purified HFC vs. active control. *Patients/Methods:* In this multinational, randomized, phase II, open-label, crossover study in 22 congenital afibrinogenemia patients aged ≥ 12 years, 70 mg kg^{-1} of new HFC (FIBRYGA, Octapharma AG) or control (Haemocomplettan® P/RiaSTAP™, CSL Behring GmbH) were administered, followed by crossover to the other concentrate. Fibrinogen activity, PK and MCF in plasma were assessed. *Results:* The concentrates were not bioequivalent for the primary endpoint, AUC_{norm} (mean ratio, 1.196; 90% confidence interval [CI], 1.117, 1.281). Remaining PK parameters ($C_{maxnorm}$, IVR, $t_{1/2}$, MRT) reflected bioequivalence between concentrates, except for clearance (mean ratio, 0.836; 90% CI, 0.781, 0.895) and V_{ss} (mean ratio, 0.886; 90% CI, 0.791, 0.994). Mean AUC_{norm} was significantly larger for the new HFC (1.62 ± 0.45 vs. 1.38 ± 0.47 h kg g L^{-1} mg^{-1}, $P = 0.0001$) and mean clearance was significantly slower (0.665 ± 0.197 vs. 0.804 ± 0.255 mL h^{-1} kg^{-1}, $P = 0.0002$). Mean MCF increased from 0 mm to 9.68 mm (new HFC) and 10.00 mm (control) 1-hour post-infusion (mean difference, −0.32 mm; 95% CI, −1.70, 1.07, n.s.). No deaths, thromboses, viral seroconversions or serious related adverse events occurred. *Conclusions:* Bioequivalence was not demonstrated for AUC_{norm}, clearance and V_{ss}. Larger AUC_{norm} and slower clearance were observed for the new HFC. Remaining pharmacokinetic parameters reflected bioequivalence to control. Safety profiles and

Correspondence: Flora Peyvandi, Fondazione Luigi Villa, Angelo Bianchi Bonomi Hemophilia and Thrombosis Centre, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Via Pace, 9 – 20122 Milano, Italy
Tel.: +39 02 5032 0727
E-mail: flora.peyvandi@unimi.it

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increases in clot strength were comparable between concentrates.

Keywords: afibrinogenemia; comparative study; congenital; fibrinogen; pharmacokinetics.

Introduction

Fibrinogen, also known as coagulation factor I, plays a central role in hemostasis, promoting clot formation and platelet aggregation [1]. Following bleeding and activation of the coagulation cascade the substrate fibrinogen is cleaved by thrombin, yielding fibrin monomers that polymerize and subsequently are cross-linked to form a stable, insoluble lattice with platelets, and red and white blood cells, thus impeding blood loss. In addition, fibrin itself has a significant thrombin binding potential, for which reason it was named antithrombin I [1]. Fibrinogen is the most abundant clotting factor in the human circulation, with normal concentrations usually ranging from 1.5 to 4.5 g L^{-1} [2], and has a half-life of approximately 4 days [3].

Inherited defects of fibrinogen can affect either the quantity (hypofibrinogenemia and afibrinogenemia) or the quality (dysfibrinogenemia) of circulating fibrinogen. Afibrinogenemia (i.e. lack of detectable fibrinogen in the plasma) is the rarest and most severe form and has an estimated incidence of approximately 1:1 000 000 [4]. It is often diagnosed in the neonatal period, with 85% of cases presenting with umbilical cord bleeds [5]. Patients with afibrinogenemia have a highly variable bleeding tendency that can be severe, including life-threatening bleeding and spontaneous/trauma-related bleeds [3].

When supplementing fibrinogen levels in patients with a/hypofibrinogenemia to treat or prevent bleeding, the therapeutic goal is to achieve a plasma level of 1 to 1.5 g L^{-1}, depending on the severity of the bleeding challenge [6]. In a survey based on data from 100 patients with inherited a/hypofibrinogenemia, the fibrinogen level most frequently recommended for on-demand treatment of minor bleeding was 1 g L^{-1}, whereas the target for major bleeding (e.g. CNS-related) was 1.5 g L^{-1} [6].

Human fibrinogen concentrate (HFC) is the treatment of choice for fibrinogen replacement in patients with congenital fibrinogen deficiency [7–9]. Cryoprecipitate is used for supplementation of fibrinogen in some countries; however, despite some progress in the use of solvent-detergent methods to treat cryoprecipitate mini-pools [10,11], this preparation does not routinely undergo pathogen inactivation [7,9]. Fibrinogen concentrate is considered to offer benefits over cryoprecipitate in terms of higher purity, more accurate/standardized dosing, faster speed of preparation and administration, no need for blood group matching, decreased allergic reactions and improved safety [7,9].

Currently, there is one main plasma-derived HFC indicated for use in congenital fibrinogen deficiency and that is widely available in a number of countries worldwide [12,13]. A single virus inactivation step (pasteurization) is used in the manufacture of this product [14–16].

A new HFC has been developed that undergoes two virus inactivation/elimination steps: solvent/detergent (S/D) treatment and nanofiltration. It is a freeze-dried, plasma-derived concentrate of human fibrinogen. The manufacturing process of this new product yields a highly-purified fibrinogen concentrate with no stabilizers added. This new HFC is currently undergoing evaluation in a broad clinical development program, which includes establishing the pharmacokinetics, efficacy and safety for on-demand treatment and surgical prophylaxis in adult and pediatric patients with congenital fibrinogen deficiency [17,18].

Here, we present the results of the first phase of the clinical development of this new HFC, a prospective, randomized trial comparing single-dose pharmacokinetics, efficacy and safety vs. a commercially available fibrinogen concentrate as active control in congenital afibrinogenemia patients. Our aims were to assess and compare the pharmacokinetic profiles as well as clot strength (by measuring maximum clot firmness [MCF]; ROTEM® thromboelastometry) as a surrogate measure of hemostatic efficacy.

Materials and methods

FORMA-01 (NCT01575756) was a multinational, multicenter, prospective, randomized, controlled, crossover, phase II, pharmacokinetic study, conducted in accordance with Good Clinical Practice (CPMP/ICH/135/95), the European Directive 2001/20/EC and applicable regulatory requirements, as well as the Declaration of Helsinki and national law. Twenty-two patients with congenital fibrinogen deficiency were enrolled and treated in this study. The study was conducted across 10 sites in six countries (Bulgaria [one patient], India [eight patients, three sites], Iran [seven patients, two sites], Switzerland [one patient], UK [three patients, one site] and the USA [two patients, two sites]) from June 2013 to January 2015. The study protocol received written approval from the country regulatory authorities, Independent Ethics Committees and Institutional Review Boards, in accordance with local requirements.

The trial consisted of two study periods, each lasting 45 days. Subjects were randomized to receive a single infusion of the new HFC (FIBRYGA, Octapharma AG, Lachen, Switzerland) or active control (Haemocomplettan® P/RiaSTAP™, CSL Behring GmbH, Marburg, Germany) in both study periods. Crossover was performed at the end of the first study period (Fig. 1). Randomization took place before first study drug administration and was performed using a computer-generated randomization schedule to assign the patients, in a blinded manner and in the order of their presentation across all centers, to

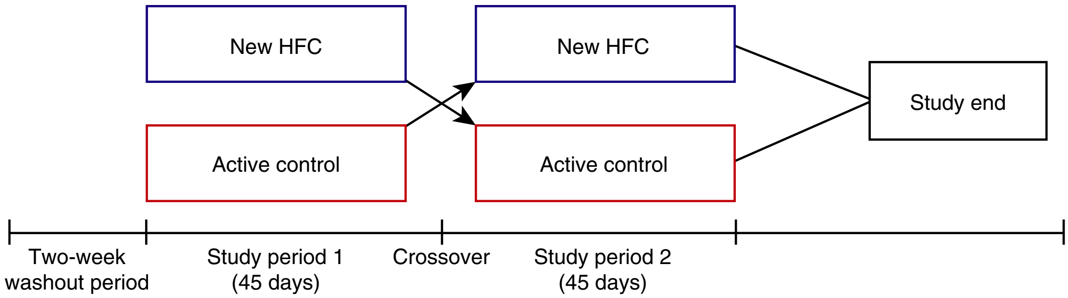

Fig. 1. The FORMA-01 study: single-dose pharmacokinetic crossover design. HFC, human fibrinogen concentrate. [Color figure can be viewed at wileyonlinelibrary.com]

treatment sequences. One central list balanced over all patients was generated. The study was open-label after randomization. The new HFC and active control were supplied in vials containing 1 g of lyophilized fibrinogen concentrate powder for reconstitution with 50 mL of water for injection. Study medication was administered as an intravenous infusion of 70 mg kg^{-1} $bodyweight^{-1}$ according to the label potency of the products.

Inclusion criteria and assessment schedule

The primary inclusion criteria were: age ≥ 12 years; documented congenital afibrinogenemia (i.e. plasma fibrinogen activity and antigen at screening below the detection limit [< 0.2 g L^{-1}]) from the central laboratory; and informed consent signed by the patient or his or her legal guardian. The primary exclusion criteria were: bleeding disorder other than congenital fibrinogen deficiency; dysfibrinogenemia; treatment with any fibrinogen concentrate or other fibrinogen-containing blood product in the 2 weeks prior to enrolment and/or subsequent study treatment; presence or history of thrombosis; acute bleeding; end-stage liver disease (i.e. Child-Pugh score B or C); planned major surgery with a need for blood transfusion during the pharmacokinetic blood-sampling period of this study; pregnancy or the intention to become pregnant during the study; and polytrauma 1 year prior to enrolment. Blood samples were collected at baseline, at 0.5, 1, 2, 4 and 8 h, and on Days 2, 3, 5, 7, 10 and 14 after infusion of medication, and all samples were tested in a central laboratory. The central laboratory for fibrinogen measurements in plasma, thrombogenicity and MCF was at Lund University, Malmö University Hospital, Malmö, Sweden, and was blinded from treatment allocation, and the laboratory for virus safety testing was INTERLAB central services–worldwide GmbH in Munich, Germany. Octapharma's quality control laboratory (Vienna, Austria) performed activity testing on the fibrinogen concentrates.

Objectives

The primary objectives were to determine the single-dose pharmacokinetic profile of the new HFC compared with the active control, and to determine MCF as a surrogate marker for hemostatic efficacy before and after administration of either product in patients with congenital afibrinogenemia. The secondary objective was to assess the safety of the new HFC in this population.

Pharmacokinetics

Fibrinogen activity was measured using a modified Clauss assay validated in the central laboratory with a limit of quantification of 0.2 g L^{-1}. The following pharmacokinetic parameters were assessed: area under the concentration-time curve (AUC), AUC normalized to the dose administered according to the measured actual potency (AUC_{norm}), *in vivo* half-life ($t_{1/2}$), incremental *in vivo* recovery (IVR), classical IVR, maximum plasma concentration (C_{max}), C_{max} normalized to the dose administered according to the measured actual potency ($C_{maxnorm}$), time to reach maximum plasma concentration (T_{max}), mean residence time (MRT), volume of distribution at steady state (V_{SS}) and clearance (CL). Incremental and classical IVRs were calculated as the maximum increase in plasma fibrinogen activity within 4 hours following infusion when compared with pre-infusion plasma fibrinogen levels.

Efficacy: clot quality (maximum clot firmness)

Thromboelastometry (ROTEM®, Tem International GmbH, Munich, Germany) was used to measure MCF. MCF is a functional parameter that depends on the activation of coagulation and the polymerization and cross-linking of the fibrin network, and is usually measured in whole blood. When measured in plasma, MCF primarily depends on the fibrinogen content of the sample and the resulting fibrin network. MCF in plasma was used as a surrogate marker of hemostatic efficacy [8,19] at 1 h after administration of either product.

All MCF measurements used frozen citrated plasma samples and the exTEM activator, and were performed in the central laboratory to minimize center-to-center variability. This methodology was used in a previous study of fibrinogen concentrate [8].

Safety

Thrombogenicity measurements included prothrombin fragments 1 and 2 (F1 + F2) and D-dimer performed prior to infusion and at 0.5, 1, 2, 4 and 8 h post-infusion on Day 1 of both study periods. Virus safety assessments included testing for HIV-1 and HIV-2, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV) and parvovirus B19 (parvo B19) by serology and polymerase chain reaction (PCR) assessments. Early allergic reactions and hypersensitivity were monitored and recorded at all post-infusion visits for the first 2 days following any treatment with study medication.

Clinical safety was assessed by monitoring vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate and body temperature) at predefined timepoints, and by monitoring laboratory parameters and adverse events (AEs) throughout the study. Hematological parameters included platelet count, hemoglobin and hematocrit. Clinical chemistry parameters included alanine aminotransferase (ALT), aspartate transaminase (AST), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase, bilirubin total, creatinine and urea, and serum electrolytes (sodium, potassium, bicarbonate and calcium).

Adverse events (AEs) and serious adverse events (SAEs) were reported from Day 1 to Day 45 in each period.

Statistical methods

Because of the limited number of patients for this ultrarare indication, no formal sample size estimations were performed. Instead, the sample size chosen on the basis of study feasibility was shown to have sufficient power for testing bioequivalence regarding AUC within a power calculation that was performed prospectively, in advance of the study start. Bioequivalence was defined as the ratio of AUCs between treatments within the bound of 0.80 to 1.25, equivalent to a mean difference on the log scale within the bound of −0.223 to +0.223. The within-subject standard deviation for AUC (on the log scale) was estimated to be 0.26. With a potential sample size of 18 evaluable patients, and assuming a 5% significance level, the study was estimated to have > 90% power, assuming a true ratio of 1 for the AUC between groups (equivalent to a mean difference of 0 on the log scale). If the true ratio was to be either 0.95 or 1.053 (equivalent to a mean difference of 0.051 on the log scale), the power would still have been over 80%.

Pharmacokinetic analyses were primarily performed in the per-protocol analysis of 21 patients. This excluded one patient who received < 90% of the planned total dose of study medication. For comparison of the pharmacokinetic parameters, 90% confidence intervals (CIs) were calculated for the mean ratio of the new HFC over active control for selected dose-independent or dose-adjusted pharmacokinetic parameters. Based on log-transformed AUC_{norm}, bioequivalence of the two products was assessed using a 2 × 2 crossover analysis of variance model (assuming independence and a normal distribution for the patient effect and error, and treatment effect and period effect as fixed effects) to test whether the 90% CI for the mean ratio of AUC_{norm} was within the range 0.8–1.25, as specified in the statistical calculations prior to the study start. The null hypothesis was non-equivalence (AUC_{norm} ratio < 0.8 or > 1.25). Following the observation of non-equivalence for some pharmacokinetic parameters, tests of the null hypothesis of no difference (ratio=1) were performed *post hoc* using ANOVA type 3 tests of fixed effects in order to further characterize any differences in the pharmacokinetic parameters between the new HFC and the active control. Descriptive *P*-values are provided accordingly.

For comparison of hemostatic efficacy, prespecified 95% CIs were calculated for the mean difference in the 1-h MCF plasma values between the two products. Safety variables were analyzed descriptively. MCF and safety were assessed in the full analysis patient dataset (*n* = 22).

Results

Patient characteristics

Twenty-seven patients were screened, of whom five were screening failures and 22 patients were treated in the study. In accordance with the inclusion criteria, patients were older than 12 years of age, with a median (range) age of 23 (12–53) years. Of the 22 patients, 14 (63.6%) were of White race and eight (36.4%) were Asian; six (27.3%) patients were between 12 and < 18 years old, and 16 (72.7%) were aged ≥ 18 years (Table 1).

Table 1 Demographic and clinical characteristics (*n* = 22)*

Parameter	Mean	SD	Median	Range
Age at first study treatment (years)	26.0	12.8	23.0	12–53
Height (cm)	161.7	14.4	159.5	130–190
Weight (kg)	65.7	17.8	69.1	33–107
BMI (kg m^{-2})	24.9	4.8	25.6	14–34
	N	%		
Age category				
< 18 years	6	27.3		
≥ 18 years	16	72.7		
Gender				
Male	7	31.8		
Female	15	68.2		
Race				
White	14	63.6		
Asian	8	36.4		

BMI, body mass index; SD, standard deviation. *Full analysis patient dataset.

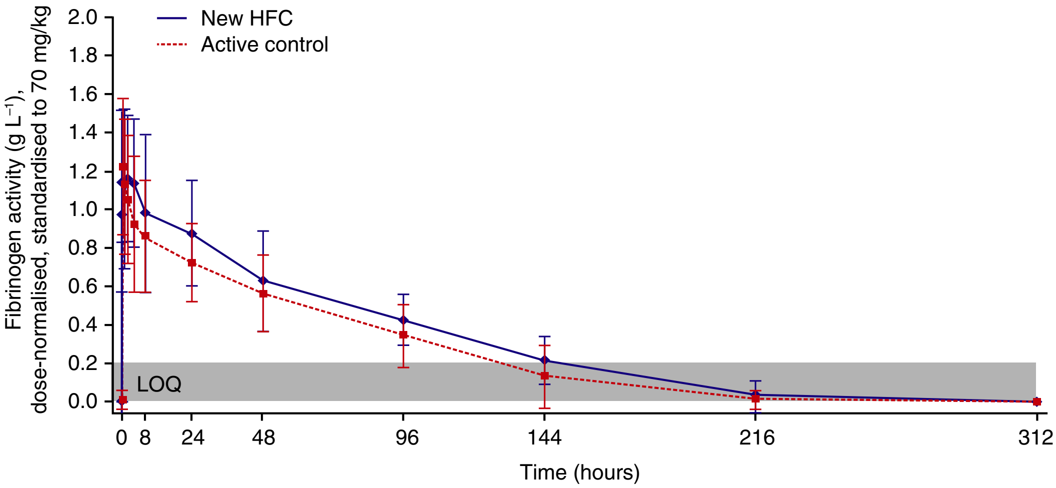

Fig. 2. Mean (± SD) fibrinogen activity (g L^{-1}) during pharmacokinetic assessment after administration of new HFC or active control (n = 21).* Fibrinogen activity plotted against time for the new HFC and active control following normalization to the dose administered and standardization to 70 mg kg^{-1}. *Per protocol patient analysis set. HFC, human fibrinogen concentrate; LOQ, limit of quantification; SD, standard deviation. [Color figure can be viewed at wileyonlinelibrary.com]

Pharmacokinetics and maximum clot firmness

The primary analyses were performed on data from 21 patients. At baseline, fibrinogen concentrations were at or below the limit of detection of the assays in all patients, as expected in this patient population. Actual mean (SD) doses of study medication administered were 76.88 (0.60) mg kg^{-1} for the new HFC and 69.74 (0.71) mg kg^{-1} for the active control. Mean fibrinogen concentration normalized to actual dose administered peaked within 2 h after administration of the new HFC (1.161 g L^{-1}) and 30 min of the active control (1.222 g L^{-1}), and for both groups decreased to pre-infusion levels by 216 h (9 days) (Fig. 2). Over the first 144 h (6 days), the mean plasma fibrinogen concentrations measured for the new HFC showed higher values than those observed with the active control (Fig. 2).

In the per-protocol analysis (n = 21), the mean ratio of the new HFC to active control for AUC_{norm} was 1.196 (90% CI, 1.117, 1.281) (Table 2). Analysis of the mean ratio for AUC_{norm} in all patients (n = 22) showed comparable results to the primary analysis (1.217; 90% CI, 1.133, 1.307). Consequently, bioequivalence was not demonstrated for the primary endpoint of the study. Bioequivalence was also not demonstrated for clearance, with mean ratio of 0.836 (90% CI, 0.781, 0.895). Furthermore, for V_{ss}, a mean ratio of 0.886 (90% CI 0.791, 0.994) was observed. For the remaining pharmacokinetic parameters, the 90% CI for the mean ratios were within the range 0.8–1.25, reflecting bioequivalence between the two products (Table 2).

Values for pharmacokinetic parameters with each study medication are shown in Table 3 (corresponding data for all patients [n = 22] are provided in Table S1). In the *post-hoc* analyses, the mean AUC_{norm} calculated for fibrinogen activity was significantly larger for the new HFC than for the active control (1.62 ± 0.45 vs. 1.38 ± 0.47 h kg g L^{-1} mg^{-1}, P = 0.0002). For the two remaining pharmacokinetic parameters that did not demonstrate bioequivalence, clearance and V_{ss}, ANOVA only showed significant differences between products in the mean value for clearance, which was significantly slower for the new HFC compared with the active control (0.665 ± 0.197 vs. 0.804 ± 0.255 mL h^{-1} kg^{-1}, P = 0.0002). These data indicate that the new HFC was

Table 2 Ratios of new HFC over active control for AUC and other pharmacokinetic parameters based on fibrinogen activity measured in plasma samples (n = 21)*

Parameter	Mean ratio†	90% CI of mean ratio‡
AUC_{norm}	1.196	1.117, 1.281
AUC	1.319	1.232, 1.413
$C_{maxnorm}$	0.989	0.927, 1.055
C_{max}	1.091	1.023, 1.162
Incremental IVR	1.005	0.939, 1.075
Classical IVR	0.972	0.903, 1.045
$t_{1/2}$	1.080	0.954, 1.224
MRT	1.061	0.944, 1.192
CL	0.836	0.781, 0.895
V_{ss}	0.886	0.791, 0.994

ANOVA, analysis of variance; AUC, area under the concentration-time curve; AUC_{norm}, area under the concentration-time curve normalized to the dose administered; C_{max}, maximum plasma concentration; $C_{maxnorm}$, maximum plasma concentration normalized to the dose administered; CI, confidence interval; CL, clearance; HFC, human fibrinogen concentrate; IVR, *in vivo* recovery; MRT, mean residence time; $t_{1/2}$, half-life; V_{ss}, volume of distribution at steady state. *Per protocol patient analysis set. †Geometric mean derived from the ANOVA model on log-transformed values. ‡Tested against the null hypothesis of non-equivalence: 90% CI of the mean ratio not contained with the range 0.8–1.25.

Table 3 Pharmacokinetic parameters (fibrinogen activity) for new HFC or active control measured in plasma samples (n = 21)*

Parameter Treatment	Mean	SD	Median	Range
AUC_{norm} (h kg g L^{-1} mg^{-1})				
New HFC	1.62	0.45	1.59	0.85–2.51
Active control	1.38	0.47	1.24	0.76–2.46
AUC_{norm}, standardized to 70 mg kg^{-1} (g h L^{-1})				
New HFC	113.70	31.54	111.14	59.70–175.51
Active control	96.39	32.89	87.03	53.36–172.16
$C_{maxnorm}$, kg g L^{-1} mg^{-1}				
New HFC	0.018	0.005	0.018	0.01–0.03
Active control	0.018	0.005	0.017	0.01–0.03
C_{max}, g L^{-1}				
New HFC	1.390	0.369	1.360	0.83–2.16
Active control	1.265	0.309	1.200	0.85–1.99
C_{max}, standardized to 70 mg kg^{-1} (g h L^{-1})				
New HFC	1.266	0.338	1.236	0.75–1.96
Active control	1.271	0.312	1.217	0.85–1.99
Incremental IVR, mg dL^{-1} increase per mg kg^{-1}				
New HFC	1.787	0.458	1.766	1.08–2.62
Active control	1.770	0.442	1.700	1.21–2.84
Classical IVR, %				
New HFC	64.397	11.519	64.830	40.89–88.13
Active control	66.046	11.635	66.756	50.17–92.46
T_{max}, h				
New HFC	2.148	1.475	2.000	0.50–4.08
Active control	1.417	2.054	0.500	0.45–8.00
$t_{1/2}$, h				
New HFC	75.940	23.831	72.854	40.03–156.96
Active control	69.378	16.006	64.644	48.60–101.94
MRT, h				
New HFC	106.272	30.927	98.975	58.72–205.47
Active control	98.977	20.812	93.462	72.38–141.21
CL, mL h^{-1} kg^{-1}				
New HFC	0.665	0.197	0.630	0.40–1.17
Active control	0.804	0.255	0.804	0.41–1.31
V_{ss}, mL kg^{-1}				
New HFC	70.158	29.860	61.037	36.89–149.11
Active control	76.631	19.579	77.701	47.89–113.68

AUC, area under the concentration-time curve; AUC_{norm}, area under the concentration-time curve normalized to the dose administered; C_{max}, maximum plasma concentration; $C_{maxnorm}$, maximum plasma concentration normalized to the dose administered; CL, clearance; HFC, human fibrinogen concentrate; IVR, *in vivo* recovery; MRT, mean residence time; SD, standard deviation; $t_{1/2}$, half-life; T_{max}, time to reach maximum plasma concentration; V_{ss}, volume of distribution at steady state. *Per protocol patient analysis set.

Table 4 MCF measured in plasma samples at baseline and 1 h after infusion of new HFC or active control (n = 22)*

Concentrate	MCF at baseline (mm) Mean ± SD Median (range)	MCF at 1 hour (mm) Mean ± SD Median (range)	Change in MCF from baseline to 1 hour (mm) 95% CI
New HFC	0 ± 0 0 (0–0)	9.68 ± 2.950† 10.00 (4.0–16.0)	8.37, 10.99
Active control	0 ± 0 0 (0–0)	10.00 ± 4.353 10.50 (0.0–17.0)‡	8.07, 11.93

CI, confidence interval; HFC, human fibrinogen concentrate; MCF, maximum clot firmness; SD, standard deviation. *Full analysis patient dataset. †Relative to active control: mean difference −0.32, 95% CI −1.70, 1.07 (not statistically significant). ‡Tested MCF for one patient was 0, despite this patient displaying a significant rise in fibrinogen levels at the same time-point. Upon evaluation, this was the result of a technical issue with the sample prior to MCF analysis.

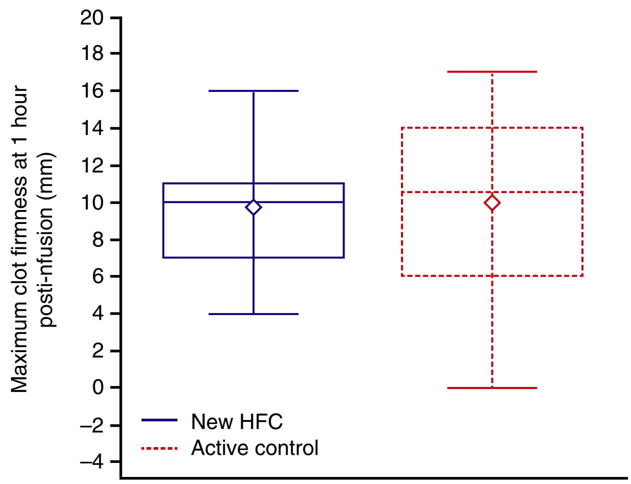

Fig. 3. Box plot of change from baseline to 1-hour after administration of new HFC or active control for MCF values in plasma samples (n = 22).* The box plot shows the median (horizontal line), mean (diamond), upper and lower quartiles (box ends), and minimum and maximum (whiskers) within 1.5-times the interquartile range. *Full analysis patient dataset. HFC, human fibrinogen concentrate; MCF, maximum clot firmness. [Color figure can be viewed at wileyonlinelibrary.com]

at least as efficacious as the active control for increasing fibrinogen activity. Furthermore, although the numbers are small, mean values for AUC_{norm} and clearance for the new HFC were similar in patients < 18 (n = 5) and ≥ 18 years old (n = 16) (AUC_{norm}, 1.573 and 1.640 h kg g L^{-1} mg^{-1}, respectively; clearance, 0.675 and 0.661 mL h^{-1} kg^{-1}, respectively).

As expected, a significant increase in mean plasma MCF from baseline (all 0.00 mm) to 1 h after administration was observed with the new HFC (9.68 mm) and with active control (10.00 mm) (both $P < 0.0001$; Table 4). The mean difference between the 1-hour post-infusion MCF values was −0.32 mm (95% CI, −1.70, 1.07); the confidence interval included zero, indicating that the difference between treatments was not significant (Fig. 3).

Safety

Adverse events generally occurred as single events in individual patients. The incidence and nature of AEs after administration of the new HFC and active control are shown for all 22 patients in Table 5.

Two AEs occurring in two patients were considered to be possibly related to the new HFC: one mild pyrexia and one mild allergic skin reaction. One severe AE

Table 5 Summary of adverse events (all patients, $n = 22$)*

	N (%) of patients, Number of events	
	New HFC	Active control
AEs	11 (50.0), 25	11 (50.0), 30
Severity of AE		
Mild	8 (36.4), 15	9 (40.9), 26
Moderate	5 (22.7), 9	3 (13.6), 4
Severe	1 (4.5), 1	0
Probably or possibly related AE	2 (9), 2	0
AE leading to withdrawal	0	0
SAE	1 (4.5), 2	0
Death	0	0

AE, adverse event; HFC, human fibrinogen concentrate; SAE, serious adverse event. *Full analysis patient dataset.

(urinary tract infection) occurred 6 days after infusion of the new HFC but was not considered to be related to treatment. One patient experienced two SAEs on the same day (abdominal pain and vaginal hemorrhage), 25 days after receiving the new HFC; these events were also not considered to be treatment related.

No deaths occurred following administration of either study medication and no severe allergic reactions, cases of thromboembolism or clinically relevant changes in prothrombin F1 + F2 and D-dimer relative to pre-infusion levels were observed. No clinically abnormal vital signs were observed during the study, and there were no seroconversions for HIV, HAV, HBV, HCV or parvovirus B19 after infusion of either product. No clinically significant abnormalities in clinical chemistry parameters were recorded with either HFC.

Discussion

This phase II, randomized trial in congenital afibrinogenemia patients represents the first phase of the clinical development of a new fibrinogen concentrate, and compared single-dose pharmacokinetics, efficacy and safety of this newly developed fibrinogen concentrate vs. a commercially available fibrinogen concentrate. The study found broadly comparable pharmacokinetic profiles of the two fibrinogen concentrates, except that the bioequivalence criteria for the primary endpoint AUC_{norm} and for the pharmacokinetic parameters clearance and V_{ss} were not reached, and demonstrated a statistically significant increase in fibrin clot quality after infusion, which translates into a potential to promote hemostasis in bleeding situations. The *post-hoc* ANOVA analyses on the pharmacokinetic parameters that did not reflect bioequivalence showed significant differences between the treatments for the area under the concentration-time curve, which was significantly larger, and for clearance, which was significantly slower, with the new HFC vs. the active control. Maximum clot firmness was significantly increased at 1 h post-infusion with both products, demonstrating similar efficacy in restoring clot strength. Both products showed a favorable safety profile with no deaths, thrombotic events, severe allergic reactions or viral seroconversions related to either treatment.

Plasma fibrinogen concentration peaked within 2 h after administration with both products, and levels achieved were well within the recommended target (1–1.5 g L^{-1}) [6] and very similar to those observed in a previous study performed with the active control (median of 1.3 g L^{-1}) [8]. The observations that bioequivalence criteria were not reached for AUC_{norm} and that the new HFC showed a significantly larger AUC_{norm} vs. the active control were surprising. Although the corresponding $C_{maxnorm}$ values were similar between the two concentrates, some differences were observed in their respective pharmacokinetic profiles. Plasma fibrinogen concentration peaked earlier after administration of active control (median T_{max} 0.5 h), whereas the peak after HFC was flatter, with a median T_{max} of 2.0 h. Following this initial phase, elimination of the two concentrates was almost parallel; $t_{1/2}$ was slightly longer with HFC vs. active control, but within the range of bioequivalence. Thus, the initial difference in the pharmacokinetic profiles could be the main determinant of the higher AUC_{norm} with HFC when compared with the active control. Another potential cause of this difference may be the slower clearance observed, which could imply enhanced persistence of the new HFC in the circulation due to increased product purity, although the mechanism for this requires further study.

As for the comparison with data published so far, most pharmacokinetic measurements were broadly in line with values reported in previous studies of fibrinogen concentrates [8,20]. Slight differences in individual parameters, for instance median $t_{1/2}$ of 77.1 h for the active control reported by a previous study [8], compared with 64.6 h observed in the current study, might be due to the variability brought about by the small sample sizes used in both studies. Nevertheless, given the robustness of the crossover design of the current study, these results should provide an accurate indication of the differences between the two products.

MCF has been established in *in vitro* and clinical studies of patients with congenital fibrinogen deficiency as a good marker for the global quality and integrity of the clotting process following administration of fibrinogen concentrate [8,19]. The rapid increases in MCF we observed after a single infusion of medication were consistent with that seen in a previous study [8]. The observed changes in MCF values also closely matched the levels expected from *in vitro* spiking experiments performed using plasma samples from individuals with fibrinogen deficiency and known amounts of exogenous fibrinogen [19]. The increase in MCF using exTEM activator in citrated plasma samples is very encouraging, but it must be kept in mind that results obtained using plasma are not equivalent to results obtained using whole blood, which is more commonly used in clinical practice, in particular in the settings of perioperative bleeding and trauma. It cannot be estimated what impact fibrinogen supplementation would have had on whole blood ROTEM analyses. Whole blood ROTEM analyses are challenging for multicenter studies in congenital afibrinogenemia; the rarity of the disease and its variability in geographic distribution, together with the need for user training and quality control, make centralized testing of plasma samples more applicable for the congenital fibrinogen deficiency setting.

Regarding safety, we observed favorable profiles for both the new HFC and the active control, the latter being an established human fibrinogen concentrate product with extensive pharmacovigilance data collected since it first became commercially available 30 years ago [21]. Adverse events generally occurred as single events in individual patients. There were no deaths, thrombotic events or severe allergic reactions in the study, nor were there seroconversions related to either treatment. These findings are in line with data reported previously, which indicate a low rate of adverse events following use of fibrinogen concentrate in populations including patients with inherited deficiency [8,21].

Our study has some limitations. With 22 patients included in the analysis, the patient cohort may be considered small, although the randomized crossover design is a rigorous test of the comparison and the study was well powered to test bioequivalence. Given that afibrinogenemia is a very rare bleeding disorder, our study population was relatively large compared with previous studies conducted in this setting [8,15,20]. There were limitations regarding the efficacy and safety analyses: the assessment of hemostatic efficacy was carried out through the surrogate measure of MCF, and the study was not powered to formally compare the safety of the two fibrinogen concentrates.

Further studies will need to incorporate direct measures of clinical efficacy. For example, the primary outcome measure of the ongoing phase III FORMA-02 study (NCT02267226) is clinical assessment of the hemostatic efficacy of the new HFC in treating acute bleeding episodes (spontaneous or after trauma) in patients aged $\geq$ 12 years with congenital afibrinogenemia or severe hypofibrinogenemia [17]. These data, together with data from the ongoing phase III FORMA-04 pediatric study (NCT02408484) [18], will provide a better understanding of how the pharmacokinetic and plasma MCF findings from the current study translate into the bleeding and perioperative setting, where prompt cessation of bleeding and restoration of hemostasis are required.

In conclusion, this randomized, controlled, single-dose, crossover trial showed that the new HFC did not reach the bioequivalence criteria for the primary endpoint of the study, AUC_{norm}. From the remaining pharmacokinetic parameters, the same was observed for clearance and V_{ss}. ANOVA analyses on these parameters showed a significantly larger AUC_{norm} and slower clearance when compared with another fibrinogen concentrate. The new HFC significantly increased clot strength 1 h after dosing, to a similar degree to the active control. The safety and tolerability profile was favorable. Additional investigations will be required to ascertain the mechanism and potential clinical impact of the favorable pharmacokinetic profile observed with this new concentrate. In the meantime, these results suggest the profile of this product to be effective for raising fibrinogen levels and correcting the clotting ability of the blood, features that are needed in the treatment and prevention of bleeding in adult and pediatric patients with congenital fibrinogen deficiency. Clinical treatment studies with the new fibrinogen concentrate are ongoing.

Addendum

C. Ross, S. Rangarajan, M. Karimi, G. Toogeh, S. Apte, T. Lissitchkov, S. Acharya, M. J. Manco-Johnson, A. Srivastava, B. Brand, B. A. Schwartz, S. Knaub and F. Peyvandi were responsible for the concept and design; analysis and/or interpretation of data; critical writing or revision of the intellectual content; and final approval of the version to be published.

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Disclosure of Conflict of Interests

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LFB. C. Ross, G. Toogeh, M. Karimi, M. J. Manco-Johnson, S. Apte and T. Lissitchkov state that they have no conflict of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Pharmacokinetic parameters (fibrinogen activity) for new HFC or active control measured in plasma samples in all patients ($n = 22$)*

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