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Review

The story of a unique molecule in hemophilia A: recombinant single-chain factor VIII

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ABSTRACT

For patients with hemophilia A, replacement of deficient factor VIII (FVIII) using plasma-derived or recombinant FVIII (rFVIII) products to restore hemostatic control can reduce bleeding complications and preserve musculoskeletal function. Despite the clinical availability of several of these products, challenges remain in the treatment of hemophilia A, the most notable of which are the risk of inhibitor development and the limited half-life of existing FVIII concentrates, which can make prophylaxis burdensome for patients. The use of recombinant protein technology may lead to novel FVIII products with improved properties. This article describes the story of a unique recombinant FVIII protein, rVIII-SingleChain, which is currently in development. In contrast to native FVIII and other commercially available rFVIII preparations, rVIII-SingleChain uses a strong, covalent bond to connect the light and heavy chains, thereby creating a stable, single-chain rFVIII. It has enhanced intrinsic stability, better integrity after reconstitution, and a higher binding affinity to von Willebrand factor. The physicochemical profile of rVIII-SingleChain and preclinical data on its activity and phamacokinetics strengthened the rationale for its clinical investigation. Available data from the AFFINITY clinical trial program are promising; indicating that it has good hemostatic efficacy when used on demand, for prophylaxis, and in the surgical setting, and is also very well tolerated. A pediatric study and an extension study are ongoing as part of the AFFINITY program.

Introduction

The standard of care for patients with hemophilia A is replacement therapy with intravenous infusion of either plasma-derived or recombinant factor VIII (rFVIII) concentrates [1]. Although several of these products are currently available [2] and included in treatment guidelines [1], improvements in product efficacy, safety, and convenience of administration continue to be a focus of rational bioengineering.

One of the principal areas of potential improvement in FVIII products is their pharmacokinetic (PK) profile. For example, coagulation factors are large proteins and tend to have a relatively short half-life; this means that frequent dosing is needed to maintain therapeutic concentrations [3]. Prophylactic treatment with currently available FVIII products necessitates intravenous injections up to three times a week [1]. Increasing the half-life of a FVIII product may permit a less frequent dosing regimen, making therapy more convenient for patients and clinicians alike, and potentially improving compliance [3]. It also has the potential to increase the uptake of prophylactic therapy, with the associated benefit of improved joint health [4]. Although prolonging the half-life of a product is a clear research aim, there are various constraints on the methodologies that can be used to do this. For example, the biological activity of the protein needs to be maintained if any structural changes are made that affect its half-life, and any modifications that are made must not increase the risk of immunogenicity [3].

Preclinical profile of rVIII-SingleChain

Recent advances in coagulation factor therapy include the development of a unique recombinant single-chain FVIII protein, rVIII-SingleChain. This protein is expressed in Chinese hamster ovary (CHO) cells and purified using chromatographic techniques [5]. It has been designed as a B-domain-truncated rFVIII molecule where the light and heavy chains are covalently linked, thereby forming a stable single-chain rFVIII [5]. This novel design is in contrast to other commercially available rFVIII preparations, which comprise two-chain molecules [5]. In addition, whereas the heavy and light chains in the native and other recombinant proteins are linked via a labile metal–ion bridge [6,7], the heavy and light chain segments in rVIII-SingleChain are linked by a stronger covalent bond, making them less likely to dissociate [5]. This is supported by an analysis of rVIII-SingleChain using size-exclusion high-performance liquid chromatography, which revealed one sharp elution peak, indicating that rVIII-SingleChain can be isolated as a homogeneous and pure compound. In contrast, full-length rFVIII produced a pattern that included multiple smaller peaks, likely owing to the heterogeneity of the residual B-domain [5]. Together, these novel features of

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rVIII-SingleChain are likely to improve its intrinsic stability and make it easier to maintain its anticoagulant properties when it is in solution during manufacture and storage [5].

The inactive form of FVIII circulates in a non-covalent complex with von Willebrand factor (VWF), which stabilizes FVIII, prevents its premature activation and clearance, and may play a role in localizing FVIII to sites of vascular injury [8]. FVIII is then activated by thrombin or factor Xa by means of proteolytic cleavage of the light and heavy chains, and is released from VWF as activated FVIII [8]. Interestingly, rVIII-SingleChain has a higher affinity for VWF than other FVIII molecules [9]. The difference between the two products in the equilibrium dissociation constant is largely driven by the fact that rVIII-SingleChain has a higher association rate constant than full-length rFVIII [9]. From these findings, we can conclude that the binding of rVIII-SingleChain to VWF is both faster and stronger than that of full-length rFVIII [9]. Similar findings have been shown in mouse models of hemophilia A [9]. As the role of VWF is to bind FVIII and prevent premature proteolysis and clearance, tighter binding of rVIII-SingleChain to VWF may reduce its clearance rate and extend its half-life until it more closely matches that of VWF. Studies in animal models support this, showing that the half-life of rVIII-SingleChain was approximately twice that of full-length rFVIII [9].

It is essential that rVIII-SingleChain can fulfill the same function within the coagulation pathway as the native protein. With this in mind, rVIII-SingleChain has been designed so that key thrombin cleavage sites required for its activation are unchanged; the active form should therefore be structurally comparable to that produced by other commercially available preparations [5]. Preclinical data support this, showing a high degree of tyrosine sulfation in rVIII-SingleChain, including in those positions that are important for binding to VWF or thrombin [5]. Analysis of thrombin digests of rVIII-SingleChain, full-length rFVIII, and B-domain-deleted rFVIII using reverse-phase high-performance liquid chromatography showed that the main peaks of all products eluted at the same retention time, indicating similarity [5]. Further support is provided by experiments using FVIII-deficient mouse models to assess the activation of rVIII-SingleChain by thrombin, which indicate that rVIII-SingleChain has similar biological activity to that of full-length rFVIII [5]. It also has similar efficacy in reducing blood loss during tail-tip bleeding in mouse models [10]. Lastly, analysis of post-translational N-glycosylation of rVIII-SingleChain using high-pH anion exchange chromatography showed a pattern that is similar to that seen in other FVIII proteins that are expressed in CHO cells [11]. In summary, the physicochemical profile of rVIII-SingleChain is similar to that of commercially available rFVIII products in terms of post-translational modification and biological activity [5,11].

Moving on to the clinic, PK profiling has been undertaken to determine whether rVIII-SingleChain exhibits the PK properties that would be expected given its molecular structure and preclinical data. Subsequent evaluation of the effect of rVIII-SingleChain on clinical outcomes will provide insights into whether this novel protein can improve patient management in hemophilia A. Both the PK profiling and the clinical evaluation are part of the AFFINITY clinical trial program, which is now well underway and is already providing insights into the use of rVIII-SingleChain in the clinical setting.

The AFFINITY clinical trial program: design

The AFFINITY clinical trial program was designed as a large interconnected series of Phase I/III studies to evaluate the efficacy, safety, and PK of rVIII-SingleChain. Participants include previously treated adults, adolescents, or children aged 12–65 years with severe hemophilia A [10]. The first phase of the program is divided into three parts, followed by a Phase III pediatric trial and an extension trial.

AFFINITY study in adults and adolescents

Part 1 of the study characterized the PK profile and short-term safety of a single dose of rVIII-SingleChain compared with a recombinant full-length rFVIII (octocog alfa; Advate®). Approximately 30 previously treated patients aged ≥18 years received one intravenous infusion of octocog alfa at 50 IU/kg and PK was measured over 72 hours. After a 4-day washout period, PK analysis was repeated after an intravenous infusion of rVIII-SingleChain [12]. Blood samples for PK analysis were collected pre-dose and up to 72 hours after dosing.

Patients who completed Part 1 of the study could then enter Part 2, in which they received multiple doses of rVIII-SingleChain as on-demand or prophylactic treatment. The results of Parts 1 and 2 informed the treatment dose and schedule for Part 3 of the study.

In Part 3, additional patients, including adolescents (12 to <18 years) and adults (18–65 years) who were receiving rVIII-SingleChain as on-demand or prophylactic treatment were included to provide a larger dataset for efficacy and safety analyses, with a subset of these patients also undergoing a single-dose PK assessment, as required by the European Medicines Agency [10]. A subgroup analysis of 13 participants who underwent elective surgery was included in the study design so that data could be collected on rVIII-SingleChain efficacy in the challenging perioperative setting [13]. After completion of Parts 1–3 of the study, eligible patients could enter the extension study in which they continue to receive treatment with rVIII-SingleChain [10]; this will provide long-term safety data. Parts 1–3 of the AFFINITY study have now been completed, and the extension study is ongoing [14].

AFFINITY study in children

In addition to the study described above, a Phase III, open-label study is ongoing in pediatric patients with severe hemophilia A [15]. Two groups are included in the study: one of previously treated patients who are 6 to <12 years old, and one of patients aged 0 to <6 years who have had over 50 exposure days with a previous FVIII product. Each group will include at least 25 patients, and will receive either on-demand or prophylactic treatment with rVIII-SingleChain at a dose decided by the investigator. The primary outcome measure in the study is rate of treatment success, defined as an investigator rating of 'excellent' or 'good' for hemostatic efficacy on a four-point scale of excellent, good, moderate, or poor/no response. Secondary outcome measures include annualized bleeding rate, proportion of bleeding episodes requiring multiple infusions to achieve hemostasis, rVIII-SingleChain consumption, and PK parameters of rVIII-SingleChain [15]. Study enrollment is now complete and data for the primary outcome measure are expected soon.

Further clinical studies are planned, including a study in previously untreated patients to assess the rate of inhibitor development with rVIII-SingleChain; it is hoped that this study will be particularly interesting, given the unique structure of rVIII-SingleChain and its enhanced binding to VWF [10].

The AFFINITY clinical trial program: preliminary results

PK profile of rVIII-SingleChain

A preliminary PK analysis has been carried out combining available data on rVIII-SingleChain from the two AFFINITY studies described above, i.e. data from adults (≥18 years old), adolescents (12 to <18 years old), and pediatrics (0 to <12 years old) [16]. In adults and adolescents, PK samples were collected pre-dose and up to 96 hours post-dose; in pediatrics, sampling was performed pre-dose and up to 48 hours post-dose. Early findings demonstrate favorable PK. As expected, mean half-life was shorter and mean clearance was greater in pediatrics compared with adults and adolescents.

Efficacy and safety of rVIII-SingleChain in adults and adolescents

Overall, 175 patients were enrolled in Parts 2 and 3 of the study, of whom 173 received treatment with rVIII-SingleChain (27 as on-demand and 146 as prophylactic therapy) [17]. The total number of exposure days in the study was 14,293; 120 patients were treated for more than 50 exposure days. During that time, 830 bleeds were treated, and the hemostatic efficacy of rVIII-SingleChain was rated as excellent or good in 94% of cases. With regard to dosing, 6% of patients who received prophylaxis were dosed every other day, 54% were dosed three times per week, 32% were dosed twice per week, and 8% followed other dosing regimens. The median annualized bleeding rate in those receiving prophylaxis was low. No inhibitor development was observed during the course of the study. In summary, these data indicate that rVIII-SingleChain is efficacious when used on-demand or as prophylaxis, and has a good safety profile.

Efficacy and safety of rVIII-SingleChain in surgical prophylaxis

The subgroup analysis that investigated the safety and efficacy of rVIII-SingleChain in the perioperative setting included 13 patients who underwent 16 major surgeries: wisdom teeth extraction, abdominal hernia repair, elbow replacement, ankle arthroplasty, five knee replacements, cholecystectomy, lengthening of the Achilles tendon combined with toe straightening, three circumcisions, surgery on an ankle fracture, and ankle hardware removal [13]. Administration of rVIII-SingleChain was via bolus infusion in eight cases and via continuous infusion in the remaining eight cases; dosing was according to the World Federation of Hemophilia guidelines and tailored to the individual patient's PK profile. On a four-point scale from excellent to poor/no response, hemostatic efficacy of rVIII-SingleChain during surgery was rated as excellent in all cases except for one, in which it was rated good. No related adverse events or serious adverse events were observed during the peri-surgical period [13].

Conclusions

The development of rVIII-SingleChain has included a comprehensive preclinical evaluation during which several unique features were highlighted and which may translate into improved clinical care for patients with hemophilia A. These include an improved PK profile, with the potential for less frequent dosing. Available data from the AFFINITY clinical trial program are promising so far, demonstrating that rVIII-SingleChain has a lower clearance, a greater area under the curve, and a longer half-life compared with full-length rFVIII. Clinical studies indicate that it has good hemostatic efficacy when used on-demand, for prophylaxis, and for surgical procedures. Furthermore, rVIII-SingleChain appears to be very well tolerated. The AFFINITY pediatric and extension studies are ongoing and further data are eagerly awaited.

Abbreviations

AUC, area under the curve; CHO, Chinese hamster ovary; FVIII, factor VIII; IR, incremental recovery; PK, pharmacokinetic; rFVIII, recombinant factor VIII; rVIII-SingleChain, recombinant single-chain factor VIII; VWF, von Willebrand factor.

Conflict of interest statement

The author has received research support from CSL Behring, has participated as a member of the scientific advisory board for Amgen, Baxter, Bayer, Boehringer Ingelheim, CSL Behring, GSK and Pfizer, and has received travel support from CSL Behring.

References

[1] Srivastava A, Brewer AK, Mauser-Bunschoten EP, Key NS, Kitchen S, Llinas A, et al. Guidelines for the management of hemophilia. Haemophilia 2013; 19: e1–e47.
[2] Farrugia A. Guide for the assessment of clotting factor concentrates. Montréal, World Federation of Hemophilia; 2008. Available at: http://www1.wfh.org/publication/files/pdf-1221.pdf. Accessed 19 April 2016.
[3] Schulte S. Innovative coagulation factors: albumin fusion technology and recombinant single-chain factor VIII. Thromb Res 2013; 131(Suppl 2): S2–S6.
[4] Fogarty PF. Biological rationale for new drugs in the bleeding disorders pipeline. Hematology Am Soc Hematol Educ Program 2011; 2011: 397–404.
[5] Schmidbauer S, Witzel R, Robbel L, Sebastian P, Grammel N, Metzner HJ, et al. Physicochemical characterisation of rVIII-SingleChain, a novel recombinant single-chain factor VIII. Thromb Res 2015; 136: 388–395
[6] Vehar GA, Keyt B, Eaton D, Rodriguez H, O'Brien DP, Rotblat F, et al. Structure of human factor VIII. Nature 1984; 312: 337–342.
[7] Eaton DL, Hass PE, Riddle L, Mather J, Wiebe M, Gregory T, et al. Characterization of recombinant human factor VIII. J Biol Chem 1987; 262: 3285–3290.
[8] Fay PJ. Activation of factor VIII and mechanisms of cofactor action. Blood Rev 2004; 18: 1–15.
[9] Zollner S, Raquet E, Claar P, Müller-Cohrs J, Metzner HJ, Weimer T, et al. Nonclinical pharmacokinetics and pharmacodynamics of rVIII-SingleChain, a novel recombinant single-chain factor VIII. Thromb Res 2014; 134: 125–131.
[10] Bensen-Kennedy D. Bringing new therapy options to the hemophilia community. Thromb Res 2013; 131(Suppl. 2): S15–S18.
[11] Schmidbauer S, Robbel L, Sebastian P, Witzel R, Grammel N, Metzner HJ, et al. Physicochemical characterization of recombinant single-chain factor VIII (RVIII-singlechain). J Thromb Haemost 2015; 13(Suppl. 2): 561 (Abstract PO146-TUE).
[12] Pabinger I, Klamroth R, Valentino L, Gill J, Levy H, Samuels R, et al. Pharmacokinetic results from a Phase I/III study of a novel recombinant single-chain Factor VIII (rVIII-SingleChain) compared to octocog alfa in severe hemophilia A patients. J Thromb Haemost 2013; 11(Suppl. 2): 712 (Abstract PB 2.55-4).
[13] Djambas Khayat C, Mahlangu J, Leisinger C, P'ng S, Santagostino E, Fernandez FL, et al. Efficacy and safety of RVIII-singlechain in surgical prophylaxis. J Thromb Haemost 2015; 13(Suppl. 2): 602 (Poster).
[14] ClinicalTrials.gov. An open-label safety and efficacy study of recombinant FVIII in patients with severe hemophilia A. Available at: https://clinicaltrials.gov/ct2/show/NCT02172950. Accessed 14 January 2016.
[15] ClinicalTrials.gov. A pharmacokinetic, efficacy, and safety study of recombinant Factor VIII Single Chain (rVIII-SingleChain) in children with severe hemophilia A. Available at: https://www.clinicaltrials.gov/ct2/show/NCT02093897?term=NCT02093897&rank=1. Accessed 14 January 2016.
[16] Mahlangu J, Lepatan LM, Vilchevska K, Oldenburg J, Stasyshyn O, Fischer K, et al. rVIII-singlechain pharmacokinetics in adults, adolescents and children. J Thromb Haemost 2015; 13(Suppl. 2): 603 (Abstract PO262-TUE).
[17] Mahlangu J, Kuliczkowski K, Stasyshyn O, Skotnicki A, Kasinova M, Kennedy DB, et al. RVIII-Singlechain, results of the pivotal phase I/III PK, efficacy and safety clinical trial in adults and adolescents with severe hemophilia A. J Thromb Haemost 2015; 13(Suppl. 2):86 (Abstract LB008).