Drug Design, Development and Therapy

Dovepress
open access to scientific and medical research

Open Access Full Text Article

LETTER

A new recombinant factor VIII: from genetics to clinical use

This article was published in the following Dove Press journal:
Drug Design, Development and Therapy
23 July 2015
Number of times this article has been viewed

Christoph Kannicht[1]
Guido Kohla[1]
Maya Tiemeyer[2]
Olaf Walter[3]
Helena Sandberg[4]

[1]Octapharma Biopharmaceuticals GmbH, Molecular Biochemistry, Berlin, Germany; [2]Octapharma Biopharmaceuticals GmbH, Heidelberg, Germany; [3]Octapharma AG, Lachen, Switzerland; [4]CoaBio AB, Bromma, Sweden

Dear editor

The December 2014 issue of *Drug Design, Development and Therapy* included a review article by Santagostino entitled "A new recombinant factor VIII: from genetics to clinical use".[1] The article provided a timely review of recent advances and developments in the treatment of hemophilia A with recombinant factor VIII (rFVIII).[1] However, when reviewing licensed rFVIII products, Santagostino[1] did not include Human-cl rhFVIII (simoctocog alfa, Nuwiq®).[2–4] Nuwiq® is a new-generation rFVIII protein produced in HEK 293 F cells that was approved by the European Medicines Agency in July 2014 for the prevention and treatment of bleeds in hemophilia A patients of all ages.[5]

Santagostino[1] described the gradual improvements made to rFVIII production/formulation and how these have coincided with the introduction of first-, second-, and third-generation rFVIII products, particularly in relation to the elimination of production-related additives from animal/human sources and viral removal/inactivation. These developments were summarized in Table 1 of the article,[1] which is adapted here with an additional row providing the respective information for Nuwiq® (Table 1).

The Nuwiq® production process is entirely free of additives of animal or human origin.[2] In addition, the purification process for Nuwiq® has incorporated technological advances into a multi-step process involving one centrifugation, two filtration, and five chromatography steps, including two dedicated virus clearance steps (solvent/detergent treatment and 20 nm nanofiltration).[2]

Santagostino[1] further described the protein structure of FVIII and the importance of post-translational modifications, importantly pointing out that "sulfation is required for full activity of FVIII" and that "glycosylation influences stability and modulates immunogenic properties". With respect to sulfation, Santagostino focused on sulfation of tyrosine 1680, which is a prerequisite for complex formation with VWF and influences the half-life of FVIII in the circulation.[1] Table 3 of the article[1] cited mass spectrometry data reported by Kannicht et al[4] relating to non-sulfated Tyr1680 in hamster-derived rFVIII products, but did not report data for Nuwiq® from the same article,[4] which indicated that the amount of non-sulfated Tyr1680 present in Nuwiq® was below the level of detection. In addition, Sandberg et al[3] reported a higher VWF-binding affinity for Nuwiq® compared with Advate®, Kogenate® or ReFacto®. Table 3 of the review by Santagostino[1] is adapted here with an additional row providing the respective information for Nuwiq® (Table 2).

With respect to glycosylation, Santagostino[1] focused on the glycosylation patterns of turoctocog alfa (NovoEight®) and concluded that:

Correspondence: Christoph Kannicht
Octapharma Biopharmaceuticals GmbH, Molecular Biochemistry, Walther-Nernst-Str 3, D-12489, Berlin, Germany
Tel +49 30 8322 7360
Fax +49 30 8410 9862
Email christoph.kannicht@octapharma.com

Table 1 Licensed recombinant factor VIII products

Generation	Product (manufacturer)	FVIII	Cell line	Culture medium	Stabilizer	Purification/viral inactivation
First	Recombinate® (Baxter BioScience)	Full-length	CHO	Bovine serum albumin	Human albumin	IAC/IEC
Second	Kogenate® FS (Bayer Healthcare)	Full-length	BHK	Human plasma protein solution	Sucrose	IAC/IEC/SD/UF
Second	Helixate® FS (CSL Behring)	Full-length	BHK	Human plasma protein solution	Sucrose	IAC/IEC/SD/UF
Third	Advate® (Baxter Healthcare)	Full-length	CHO	None	Trehalose	IAC/IEC/SD
Third	Xyntha/ReFacto® AF (Pfizer)	B-domain-deleted	CHO	None	Sucrose	IAC/IEC/SD/NF
Third	Turoctocog alfa (Novo Nordisk)	B-domain-truncated	CHO	None	Sucrose	IAC/IEC/SD/NF/SE
New	Nuwiq® (Octapharma AG)	B-domain-deleted	HEK	None	Sucrose/ arginine	IAC/IEC/SD/NF/SE

Note: Copyright © 2014. Dove Medical Press. Adapted from Santagostino E. A new recombinant factor VIII: from genetics to clinical use. *Drug Des Devel Ther*. 2014;8: 2507–2515.[1] Additional data added for Nuwiq®.[2–4]
Abbreviations: FVIII, factor VIII; IAC, immunoaffinity chromatography; IEC, ion exchange chromatography; NF, nanofiltration; SD, solvent/detergent treatment; SE, size exclusion; UF, ultrafiltration.

Table 2 Levels of non-sulfated tyrosine in rFVIII

Product	Origin	Non-sulfated tyrosine 1680 (%)		
		LC-MS/MS	LC-MS/MS	MS-FT
Turoctocog alfa	CHO	–	–	Below detection limit
Full-length, third-generation rFVIII	CHO	2.6–16.7	5.0–8.0	>9.0
Full-length, second-generation rFVIII	BHK	1–6.5	1.5	–
BDD third-generation rFVIII	CHO	4.5–13.9	4.0–5.0	–
Simoctocog alfa, BDD new-generation rFVIII	HEK	–	Below detection limit	–

Notes: Levels of non-sulfated tyrosine in rFVIII included Advate® (full-length third-generation rFVIII), Kogenate FS® (full-length second-generation rFVIII), and Xyntha/ReFacto AF® (B-domain-deleted third-generation rFVIII). The sulfated form dominated for all proteins, with the proportion of non-sulfated tyrosine 1680 being highest for some third-generation rFVIII products. A small peak for the non-sulfated isoform was also observed for second-generation rFVIII, while no non-sulfated species were detected for turoctocog alfa or Nuwiq® (B-domain-deleted new-generation rFVIII). Below detection limit means negligible signal, <1%, or <0.5%, trace. Copyright © 2014. Dove Medical Press. Adapted from Santagostino E. A new recombinant factor VIII: from genetics to clinical use. *Drug Des Devel Ther*. 2014;8:2507–2515.[1]
Abbreviations: BDD, B-domain-deleted; LC-MS/MS, liquid chromatography tandem mass spectrometry; rFVIII, recombinant factor VIII; MS-FT, mass spectrometry high resolution Fourier transform scan.

> The oligosaccharide structures of the novel rFVIII [NovoEight®] and plasma-derived FVIII are very similar, with mainly small, quantitative differences, and heterogeneous glycosylation is present in both products.

Comparable glycosylation of Nuwiq® and plasma-derived FVIII has also been reported.[4] It is well documented that potentially antigenic non-human glycan epitopes, such as *N*-glycolylneuraminic acid (Neu5Gc) or Gal-α1-3Galβ1-(3)4GlcNAc-R (α-Gal), are present in recombinant products derived from hamster cells.[4,6,7] Thus, a comparison of these epitopes in rFVIII products derived from hamster cells might have been of interest to your readers. As Nuwiq® is produced in a human cell line, Neu5Gc or α-Gal are not present.[4]

In summary, the Santagostino article[1] was a welcome addition to the literature that provided a timely update on recent advances and developments in rFVIII treatment of hemophilia A. However, the omission of data for the new-generation human cell derived rFVIII, Nuwiq®, which have been summarized in this letter, was a major limitation of the article.[1]

Disclosure

Christoph Kannicht, Guido Kohla, Maya Tiemeyer, Olaf Walter are employees of Octapharma. Helena Sandberg is a former employee of Octapharma. Editorial assistance was provided by nspm ltd, Meggen, Switzerland, with financial support from Octapharma.

References

1. Santagostino E. A new recombinant factor VIII: from genetics to clinical use. *Drug Des Devel Ther.* 2014;8:2507–2515.
2. Casademunt E, Martinelle K, Jernberg M, et al. The first recombinant human coagulation factor VIII of human origin: human cell line and manufacturing characteristics. *Eur J Haematol.* 2012;89(2):165–176.

3. Sandberg H, Kannicht C, Stenlund P, et al. Functional characteristics of the novel, human-derived recombinant FVIII protein product, human-cl rhFVIII. *Thromb Res.* 2012;130(5):808–817.
4. Kannicht C, Ramstrom M, Kohla G, et al. Characterisation of the post-translational modifications of a novel, human cell line-derived recombinant human factor VIII. *Thromb Res.* 2013;131(1):78–88.
5. European Medicines Agency [homepage on the Internet]. Nuwiq simoctocog alfa (rFVIII); 2014 [updated December 19, 2014]. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002813/human_med_001781.jsp&mid=WC0b01ac058001d124. Accessed April 16, 2015.
6. Hokke CH, Bergwerff AA, van Dedem GW, et al. Sialylated carbohydrate chains of recombinant human glycoproteins expressed in Chinese hamster ovary cells contain traces of N-glycolylneuraminic acid. *FEBS Lett*. 1990;275(1–2):9–14.
7. Hironaka T, Furukawa K, Esmon PC, et al. Comparative study of the sugar chains of factor VIII purified from human plasma and from the culture media of recombinant baby hamster kidney cells. *J Biol Chem.* 1992;267(12):8012–8020.