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Review Article

Challenges for new haemophilia products from a manufacturer's perspective

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ABSTRACT

The development of new and improved therapeutic options for the management of haemophilia is a great challenge for both physicians and manufacturers. After factor VIII concentrates became widely available, progress in medicine and advances in biotechnology led to the development of virus-inactivated, plasma-derived products of high purity and recombinant products, which are currently further improved regarding an extended half-life, potentially allowing for effective prophylaxis with reduced dosing frequency and hopefully less immunogenicity. This article describes some of the challenges that were experienced by the manufacturer during the development of the high-purity, plasma-derived factor VIII concentrate, Beriate® P, which, after implementation of a nanofiltration step in its manufacture, is now designated as Beriate®; it also outlines the challenges and achievements to date with half-life extended products such as the recombinant fusion proteins linking coagulation factor VIIa (rVIIa-FP) and factor IX (rIX-FP) with albumin.

Contents

Introduction

In the late 1960s, factor VIII (FVIII) concentrates became available for the treatment of patients with haemophilia A. They were purified from cryoprecipitate, which was obtained from large pools of donated plasma. These products, which were manufactured in the USA, were very well received as they provided patients with an effective therapy for the first time, especially in the home-care setting. However, the specific activity of these products was quite low (<5 IU FVIII/mg protein); tolerability was lower and the frequency of allergic reactions was higher than what we see with today's products. Moreover, virus transmission of human origin, such as hepatitis B and C and human immunodeficiency virus occurred with these early products as no measures for virus inactivation had been implemented. In the following years, concentrates of intermediate purity were developed (e.g. Faktor VIII® Behringwerke and Haemate® [without pasteurization]), which displayed a much better tolerability profile, but with an unchanged

Abbreviations: CHO, Chinese hamster ovary; DEAE, diethylamino ethanol; FVIIa, activated factor VII; FVIII, factor VIII; FIX, factor IX; FXI, factor XI; HEK, human embryonic kidney; HS, heat sterilized; NHP, natural human plasma; PCR, polymerase chain reaction; QAE, quaternary aminoethyl; rFVIIa, activated recombinant factor VII; rFIX, recombinant factor IX; rVIIa-FP, recombinant fusion protein linking coagulation factor VIIa with albumin; rIX-FP, recombinant fusion protein linking coagulation factor IX with albumin; TF, tissue factor; VWF, von Willebrand factor.

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risk of virus transmission. At that time, the greatest challenge for the manufacturers of plasma proteins was to develop techniques for effective inactivation of blood-borne viruses [1]. Heimburger, Kumpe and colleagues from the German company Behringwerke (predecessor of CSL Behring) successfully developed a method to pasteurize FVIII in aqueous solutions, a method that effectively inactivated both enveloped and non-enveloped viruses [2]. Haemate® P, the first pasteurized and virus-inactivated FVIII product (FVIII/von Willebrand factor [VWF] complex), was clinically investigated in the late 1970s and registered in Germany in February 1981 under the name Faktor VIII®:HS Behringwerke. In 1985, the product's name was changed to Haemate® HS (HS = hepatitis safe) and later to Haemate® P (P = pasteurized).

Challenges of Yesterday and Today

Development of the Pasteurized, High-purity FVIII Concentrate Beriate® P

Implementation of an effective method of virus inactivation was a very important step forward in the treatment of haemophilia. Nevertheless, there were still several other desirable product improvements that were needed, such as reduction in the level of co-purifying proteins to improve purity, reduction of Isoagglutinins to enhance tolerability, higher FVIII concentration to allow for a lower reconstitution volume, stabilization without albumin and, of course, better FVIII yields for optimal use of the valuable donated plasma. Heimburger, Kumpe and colleagues also worked on these challenges and between 1984 and 1991 developed several versions of Beriate® P, a high-purity, pasteurized FVIII concentrate [3]. Table 1 shows the major milestones of this development.

The first version of Beriate® P, referred to as FVIII:C® HS (in contrast to Haemate® P, which was referred to as FVIII®:HS), was produced on a laboratory scale. Several sophisticated adsorption and chromatography steps led to a much better purification of FVIII:C, however the concentration was limited to 25 IU FVIII/mL due to the addition of albumin for stabilization. After scaling up of the manufacturing process from the laboratory to industrial production, several pre-consistency and consistency lots were produced. Unfortunately, the first FVIII batches were unstable; a finding that highlights the difficulties in obtaining a robust product in the final industrial process. To improve the stability of FVIII, Heimburger introduced an additional adsorption step. Consistency lots were then produced without any further problems, and clinical studies with Beriate® P, called FVIII:C® HS during the study phase, were subsequently conducted. The product was registered in Germany in 1990 and in Austria in 1992 under the name Beriate® HS (later changed to Beriate® P) and introduced into the market more widely starting in 1990. In the following years, Beriate® HS was further purified and stabilized with glycine and sucrose instead of albumin. In July 1991, the first version of the product was replaced by a high-purity, more concentrated Beriate® HS containing 50 IU FVIII/mL which was also stabilized without albumin; one year later the third version of Beriate® HS containing 100 IU FVIII/mL was introduced.

The next major milestone was the complete renewal of the Beriate® P production plant in 1997/98. Although it is always important to keep the production process state-of-the-art, it is paramount that changes in equipment and processing do not modify the key product characteristics. Indeed, extensive experience, knowledge and process acumen, as well as careful work, is necessary to ensure that the product quality remains unaffected. Fig. 1 shows the main steps of the Beriate® P production process without nanofiltration.

Production starts with a solution of the cryoprecipitate followed by an adsorption step using aluminium hydroxide, which removes several prothrombin factors (factors II, VII, IX and X). The next stage is a combined adsorption step using aluminium hydroxide and QAE (quaternary aminoethyl) Sepharose A-50 to further reduce other proteases that could affect the stability of FVIII. In particular, it has recently been shown that this step specifically removes a protease called factor-seven-activating protease, which rapidly degrades FVIII. The supernatant from the combined adsorption on aluminium-hydroxide/QAE Sepharose A-50 step is then stabilized with glycine and sucrose and then heated for 10 hours at 60 °C in aqueous solution (pasteurization). Thereafter, the protein solution is diluted and FVIII is purified further on DEAE (diethylamino ethanol) Sepharose. This specifically designed purification step is almost as effective as affinity chromatography, producing a highly purified FVIII product that still contains sufficient amounts of VWF to stabilize the labile FVIII molecule. The final production steps are ultrafiltration, sterile filtration, filling and lyophilization.

Table 1
Milestones in the development of Beriate® P (FVIII:C® HS during clinical studies).

Date	Milestone
1984–1985	Development of the first version of Beriate® P (25 IU FVIII/mL, stabilized with albumin)
1986–1988	Up-scaling and production of technical batches
May 1988	First consistency lot with unstable FVIII
September 1988	Consistency lot with stable FVIII
1990/1992	Registration and launch of Beriate® P in Germany and Austria
July 1991	Registration and launch of the second version of Beriate® P high-purity (50 IU FVIII/mL, stabilized without albumin)
September 1992	Registration and launch of the third version of Beriate® P high-purity (100 IU FVIII/mL, stabilized without albumin)

FVIII, factor VIII.

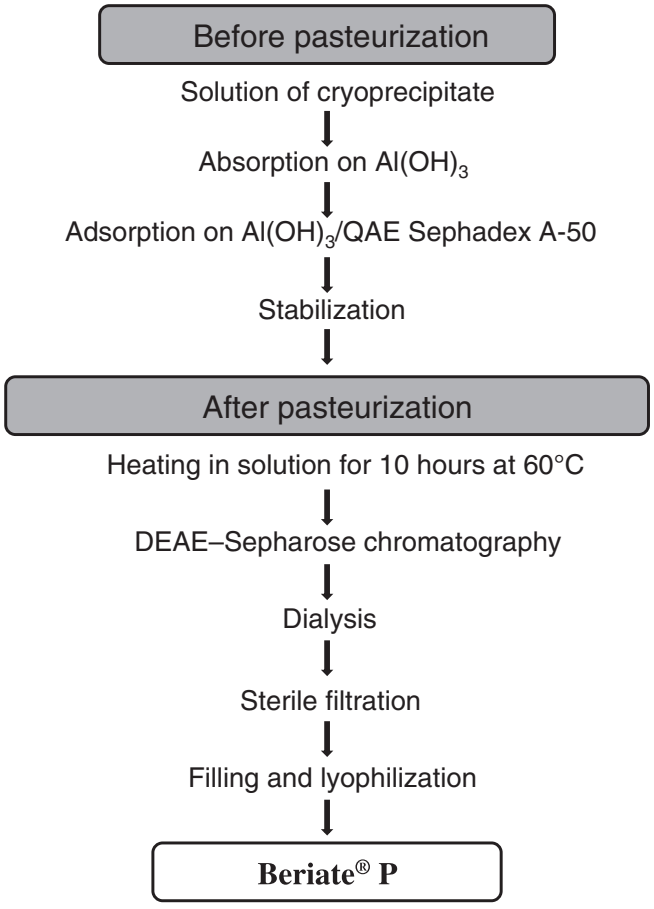

Fig. 1. Production process of Beriate® P without nanofiltration. $Al(OH)_3$, aluminium hydroxide; DEAE, diethylamino ethanol; QAE, quaternary aminoethyl.