Porcine recombinant factor VIII: an additional weapon to handle anti-factor VIII antibodies

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Abstract
This review focuses on the use of recombinant porcine factor VIII (FVIII) for the treatment of bleeding episodes in patients with severe haemophilia A complicated by inhibitors and in those with acquired haemophilia A due to the onset of anti-FVIII autoantibodies. We present the main characteristics of recombinant porcine factor VIII (FVIII) and provide a summary of the published results of the clinical studies of this novel recombinant FVIII. There is a special emphasis on how the use of recombinant porcine factor VIII (FVIII) is expected to improve the clinical management of these patients.

Keywords: haemophilia A, inhibitors, bleeding, porcine recombinant FVIII, Obizur.

Introduction
The last 30 years have seen the development of viral inactivation procedures and recombinant products that have greatly improved replacement therapy in haemophilia A. These advances have had a positive impact on patients’ health due to the widespread adoption of prophylaxis to prevent arthropathy and, therefore, improvements have also been seen in patients’ quality of life and life expectancy. Therapy for haemophilia A is now of high quality and it is probably among the most efficacious and safe treatments available for monogenic disorders. In this context, the most significant therapeutic complication of haemophilia A has become the development of alloantibodies against factor VIII (FVIII) that inhibit its coagulant activity (inhibitors). Inhibitory alloantibodies develop in up to 30-35% of patients with severe haemophilia A, usually within the first 15-20 days of treatment. They make replacement therapy ineffective, compromise patient access to a safe and effective standard of care, and put them at high risk of morbidity and mortality. The introduction of bypassing agents (the activated prothrombin complex concentrate FEIBA (Baxalta, Bannockburn, IL, USA) and the recombinant activated factor VII (Novo Nordisk, Bagsvaerd, Denmark) is an effective therapeutic strategy for bleeding episodes in patients with inhibitors. However, these drugs have lower overall haemostatic efficacy than FVIII products in haemophilia A patients without inhibitors. At the beginning of the 1970s, it was observed that the degree of cross-reactivity of anti-human FVIII antibodies with porcine FVIII was much lower than that towards the human factor (average <30%). This paved the way for the use of plasma-derived porcine FVIII, which, in the early 1980s, led to the production of Hyate:C (Speywood/Ipsen Ltd., Wrexham, United Kingdom). The lower cross-reactivity of porcine FVIII with anti-human FVIII antibodies contributed to a substantial increase in the threshold of bleeding episodes that could be handled with FVIII replacement. Besides its use in the treatment of patients with congenital haemophilia A with inhibitors, this plasma-derived porcine FVIII product was also successfully used to manage bleeding events in patients with acquired haemophilia A, a rare but often life-threatening acquired haemorrhagic condition caused by the development of FVIII autoantibodies. The rationale for the use of this product was the observation that the degree of reactivity of autoantibodies towards porcine factor VIII was even lower than that of alloantibodies. While this plasmatic product gave fewer hypersensitivity reactions than the previous less purified preparations, thrombocytopenia continued to be a frequent post-infusion occurrence due to in vivo platelet aggregation induced by the porcine von Willebrand factor (VWF) contained in the product. Unfortunately, porcine parvovirus was detected in some batches (the product was not subjected to viral inactivation steps), and this led to production of Hyate:C being interrupted. Although this virus is not pathogenic to humans, Hyate:C was withdrawn from the market in 2004. In parallel, a new porcine FVIII molecule was developed using recombinant DNA technology (Obizur [OBI-1], Baxalta), with the aim of eliminating the main limitations of the previous plasma-derived preparations, i.e. the content of VWF, other immunogenic and allergenic porcine plasma proteins, and the risk of viral contamination. Current knowledge of the development of recombinant porcine FVIII is summarised below and we also identify the...
expected advances in the clinical management of patients with anti-FVIII antibodies.

Methods
Search methods
We analysed the medical literature for published studies on porcine recombinant FVIII for treatment of haemophilia patients with inhibitors. The MEDLINE electronic database was searched for all publications in English. The Medical Subject Heading and keywords used were: "congenital haemophilia A", "acquired haemophilia", "FVIII", "inhibitors", "alloantibodies", "autoantibodies", "by-passing agents", "recombinant porcine FVIII", "plasma-derived porcine FVIII", "OBI-1", "Obizur", "bleeding episodes". We also screened the reference lists of the most relevant articles for additional studies not captured in our initial literature search. Search terms were also applied to abstracts from the latest international congresses on haematology, haemostasis and thrombosis.

Characterisation of porcine recombinant FVIII
Pharmacology
Obizur (OBI-1) is a high-purity B-domain deleted form of porcine FVIII produced by recombinant DNA technology in baby hamster kidney cells grown in a serum-free medium. It has the same domain sequence and subunit structure as human FVIII and contains a 24 amino-acid-linker comprising the first and the last 12 amino acids of the B-domain. In spite of this close homology, the significant divergence in the amino acid sequence of the immune-dominant epitopes in the A2 and C2 domains is likely to explain the lower degree of reactivity of most human anti-FVIII antibodies with porcine FVIII. OBI-1 undergoes two viral reduction/inactivation steps (solvent detergent and nanofiltration) and its formulation contains no animal-derived products. In contrast to Hyate:C, this recombinant FVIII contains no VWF, thus eliminating the risk of thrombocytopenia. OBI-1 circulates almost completely (98%) as a heterodimer, binds with high affinity to human VWF, and is activated by thrombin.

Pre-clinical studies
The immunogenicity, pharmacokinetics, safety and haemostatic efficacy of OBI-1 and Hyate:C were first compared in animal models. Overall, pre-clinical immunogenicity studies in haemophilic mice and in cynomolgus monkeys indicated that this recombinant FVIII has an immunogenicity profile similar to that of plasma-derived porcine FVIII, but with significant differences in domain recognition. Comparison of pharmacokinetic parameters in monkeys and haemophilic dogs showed that OBI-1 has a favourable pharmacokinetic profile, with higher maximum plasma concentration and area under the curve (AUC) values. In addition to the observed higher plasma recovery of recombinant FVIII compared with Hyate:C (due to a lower clearance of the former), other pre-clinical studies demonstrated the efficacy of OBI-1 in reducing the cuticle bleeding time in haemophilic dogs and blood loss in a tail-snip assay in haemophilic mice. Finally, OBI-1 also demonstrated a favourable safety and efficacy profile in canine and primate animal studies.

Clinical experience with recombinant porcine FVIII
Acquired haemophilia A
As mentioned above, the rationale for the clinical use of recombinant porcine FVIII in acquired haemophilia A is based upon its ability to achieve haemostatic plasma levels of FVIII coagulant activity thanks to the low reactivity of anti-human FVIII autoantibodies towards porcine FVIII. The efficacy and safety of OBI-1 for the treatment of bleeding episodes in patients with acquired haemophilia A was recently assessed in a prospective, open label phase II-III study. All the 28 patients enrolled who presented with severe bleeding episodes were initially treated after hospitalisation with the same dose of recombinant porcine FVIII (200 U/Kg) followed by additional doses based on the targeted FVIII activity levels and clinical responses in the recipients. The choice of the initial dose of porcine FVIII was based on the assumption that this large dose would overcome any inhibitor titre and achieve measurable FVIII levels in plasma even when inhibitor levels and the degree of antibody cross-reactivity with porcine FVIII are not known. All the 28 subjects with acquired haemophilia A met the primary end point of a positive clinical response to treatment (effective or partially effective according to the response criteria) 24 hours after the first drug dose, the majority of them (95%) responding within 8 hours of administration. Notably, the primary bleeding episode was controlled in 16 of 17 subjects who had received porcine recombinant FVIII as first-line treatment. Overall, 24 of 28 (87%) subjects achieved successful control of their bleeding episode at the time of the last dose of the recombinant agent. The median total dose infused was 1,580 U/kg, the median dose per infusion was 116 U/kg, and the median total number of infusions was 12 per patient. The median rise in FVIII activity was 203% immediately after the initial loading dose of 200 U/kg and 108% at 24 hours post infusion, thus documenting the ability of recombinant porcine FVIII to restore normal haemostasis in patients with acquired haemophilia A. No serious adverse events (including thrombosis) were recorded. The successful control of bleeding with OBI-1 in acquired haemophilia A was also
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reported in 2 independent cases of acquired haemophilia A (1 following stem cell transplantation for sickle cell disease and 1 associated with cancer)\(^{26,27}\). Thanks to these positive results, Obizur was approved by the Food and Drug Administration (FDA) and marketed in the USA for the treatment of bleeding episodes in patients with acquired haemophilia A. Although it has not yet been licensed in Italy, it is available under the conditions set out in the 1996 Law n. 648\(^{28}\).

**Congenital haemophilia A complicated by alloantibodies**

Some preliminary data on this unlicensed indication are available. In a phase I randomised, double blind clinical trial, the pharmacokinetic profile and safety of OBI-1 was compared with Hyate:C in 9 haemophilia A patients with FVIII inhibitors\(^{29}\). The subjects in a non-bleeding state were randomised to receive placebo followed by 100 U/kg of recombinant porcine FVIII (n=4 patients) or 100 U/kg Hyate:C followed by placebo (n=5). The mean C\(_{\text{max}}\) and AUC were higher for OBI-1 than Hyate:C, thus supporting the view that the recombinant product has a greater bioavailability. No OBI-1 infusion-related adverse events were recorded. Unfortunately, this study was prematurely stopped because Hyate:C was no longer available, so that the sample size was smaller than originally planned. A phase II, open label study evaluating the haemostatic activity, pharmacokinetics and safety of OBI-1 was conducted in 9 patients with congenital haemophilia A complicated by FVIII inhibitors, who were experiencing a non-life-threatening or non-limb-threatening bleeding episode\(^{30}\). A total of 25 bleeds were treated in the 9 patients with a loading dose of 200 U/kg, followed by up to 8 doses of 50-150 U/kg. All the 25 bleeding episodes were successfully treated with OBI-1 of which 20 (80%) were controlled with only one dose. No severe drug-related adverse events were observed.

**Conclusions**

From our analysis of the published pre-clinical and clinical studies, it appears that recombinant, B-domain deleted, porcine FVIII is a potentially important therapeutic option for patients with haemophilia A complicated by or caused by allo- and auto-antibodies\(^{31}\). The data from the prospective phase II-III study by Kruse-Jarres et al.\(^{25}\) demonstrated that recombinant porcine FVIII is a safe and effective treatment of bleeding in patients with acquired haemophilia A. The main attraction of this study was the demonstration that the chosen approach of a fixed dose without previously measuring the degree of cross-reactivity of the antibody with porcine FVIII is valid. This is a very important point, considering that acquired haemophilia A can have a dramatic clinical presentation requiring urgent treatment, and immediate support from specialised laboratories is not always available such emergency situations. The very large dose was chosen (200 IU FVIII/kg) and the majority of patients with acquired haemophilia reached unnecessarily high levels of plasma FVIII. Further studies should be aimed at evaluating whether or not a smaller fixed dose (e.g. 100 IU/Kg) is as useful in terms of plasma FVIII levels and clinical efficacy.

Data generated from phase I-II studies in acquired haemophilia should stimulate study of the safety and efficacy profile of OBI-1 also in the clinical setting of congenital haemophilia A complicated by inhibitors. This recombinant agent could have the potential role of achieving and consolidating haemostasis through gaining measurable plasma levels of FVIII in patients with a suitable degree of cross-reactivity, particularly in cases of life-endangering bleeding episodes or major surgery. This targeted approach involves measuring the degree of cross-reactivity of the anti-human FVIII alloantibody with recombinant FVIII in order to establish the feasibility of this therapeutic approach and the most appropriate dosage. In the past, such assays were developed to promote the optimal use of Hyate:C\(^{6,32}\), and they can be re-visited and suitably modified to use with OBI-1.

**The Authors declare no conflicts of interest.**

**References**