

## A Look at the Future Hemophilia A Treatment

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**Abstract.** The current treatment of patients with hemophilia A is safer and more effective than the previous one. Prophylactic substitution involves repeated intravenous administration of plasma-derived factor VIII or recombinant factor VIII products, with inconveniences and possible adverse effects. The occurrence of inhibitors requires the administration of activated prothrombin complex concentrate or activated factor VII - an expensive treatment. The immune tolerance induction is the ideal treatment for patients with high titres of inhibitors - the only potential way to eliminate inhibitors and very expensive. For these reasons, the medical world is interested in the advances that scientific research is doing in the field of new molecules without the inconveniences of current substitution therapy and which could replace it in the future. The purpose of this article is to briefly review the new therapeutic possibilities for patients with hemophilia A, which can prevent potential extraarticular bleedings, avoid the occurrence of inhibitors, and have as few adverse effects as possible.

### Introduction

The prophylaxis and the treatment of bleeding episodes of patients with hemophilia A without inhibitors is currently made with plasma-derived factor VIII or recombinant factor VIII products. Plasma-derived factor VIII is less immunogenic as recombinant factor VIII products, but less sure regarding the possibility of transmission of some infectious biological agents (e.g., some viruses, including those that are still unknown, or prions). Large-scale recombinant factor VIII production has increased the number of patients currently benefiting from prophylactic substitution, a desirable attitude for the prevention of disabling arthropathies and dangerous haemorrhagic accidents. It appears, however, that the risk of occurrence of inhibitors is higher as the purity of factor VIII increases. It is certain that inhibitors occur more frequently at the first doses (therefore, they occur more frequently in children) or higher doses of factor VIII, but may occur (less frequently) in the elderly, too. The current disputes in the literature on the possible increase of patients who develop inhibitors due to recombinant factor VIII products have not been solved, but it should be underlined that the incidence of high titre inhibitors is stable over time, and this is most important, as only they raise difficult treatment issues.

The appearance of inhibitors requires a bypass therapy: activated prothrombin complex concentrate or activated factor VII. The immune tolerance induction is the ideal treatment for patients with high titres of inhibitors - the only potential way to eliminate inhibitors. This is very expensive. Corticosteroids, immunosuppressive drugs or rituximab may be therapeutic alternatives, but not as effective.

The substitution treatment requires 2 or 3 weekly factor VIII administrations that are given intravenously. These repeated injections for a long time (even decades) are not devoid of potential adverse effects and inconvenience related to drug administration. In addition, some patients may develop anti-factor VIII antibodies (“inhibitors”).

Given the current treatment limits, I decided to make a literature review on new therapies that can improve therapeutic outcomes and increase the quality of life of haemophilic patients. I selected the articles published in PubMed and PubMed Central databases from July 2015 to December 2017.

The used search terms included "hemophilia A", "inhibitors", "plasma-derived factor VIII" and "recombinant factor VIII".

### **Therapeutic Solutions for the Future**

A purpose of future therapies is to produce novel haemostatic agents with lesser immunogenicity, with the possibility of effective administration in a smaller number of doses, or even making products that avoid the need for substitution therapy with coagulation factors [1]. New therapeutic means for hemophilic patients with inhibitors may therefore consist in the introduction of nonfactor replacement strategies [2]. Thus, haematologists will probably be able to use in the future drugs that increase thrombin generation by bypassing the inhibitor, or products that act by natural anticoagulant inhibition [3], as those that counteract the tissue factor pathway inhibitor [2] with a humanized monoclonal antibody (concizumab) [4, 5]. The annihilation of antithrombin 3 may be obtained using aptamers, antibodies, or genetic engineering techniques, like small RNA interference technology [2] [small interfering RNA (siRNA), alnylam initiates fitusiran (ALN-AT3)] which suppress hepatic antithrombin production by post-transcriptional gene silencing [4]. The concomitant administration of fitusiran and high-dose factor VIII, contrary to the recommendations of the first clinical trial with this new drug to avoid high coagulation factors, was associated with a fatal thrombosis [6]. Subsequently, the FDA allowed to start the trial again but at lower dosages. In addition, bispecific antibody technology (asymmetric antibody given subcutaneously which interacts with activated factor IX and factor X) may be used to mimic the function of coagulation factor VIII [4, 2], such as emicizumab [4] or ACE910 [5]. Among the severe side effects of Emicizumab are: five deaths, one of which is for severe intestinal bleeding. Other objectives are: achieving less immunogenic factor VIII products and modifying B-cell epitopes to decrease the attachment of factor VIII molecules to antibodies or memory B lymphocytes [7]. Unfortunately, the immunogenicity of new products administered in previously untreated patients is not very well known; so far, we have data on the development of inhibitors only in previously treated patients.

#### **Extended half-life factor VIII products**

Until then, extended half-life factor VIII products allow for increased patient compliance versus prophylaxis by rarely administering the product (once given twice a week). They are safe and patients treated so far have not developed inhibitors, according to published studies. The incidence of inhibitors after administration of these products to previously untreated patients is yet unknown [8], but some authors claim they can increase the incidence of inhibitors [9]. Indeed, a more rare administration of substitution therapy in hemophilic patients may be achieved using longer-acting FVIII concentrates, but genetic or chemical modification of the protein may increase the risk of inhibitors occurrence, due to its higher immunogenicity. A transgenic mouse line into which human F8 cDNA has been introduced and which is immunologically tolerant can be used for immunological testing of new factor VIII products [9]. Factor VIII DNA formulated in chitosan nanoparticles with a size of 200-400 nm is able to protect DNA against pH degradation and endonuclease activity. This new medication, given orally and repeatedly, allowed a sustained increase in factor VIII activity without producing detectable inhibitors [10].

#### **The high-purity human factor VIII/von Willebrand factor concentrate**

The thrombin-generating test may be used to guide the treatment with bypassing agents. In addition, the description of inhibitors can be done with epitope mapping. So guided, the treatment with high-purity human factor VIII/von Willebrand factor concentrate has led to a decrease of inhibitor titer against the C2 domain in some published cases [11].

#### **The B-domain deleted factor VIII products**

Some patients with unsatisfactory response to bypassing agent therapy could be treated with a recombinant porcine factor VIII with B-domain-deleted product. In a retrospective study done on

7 patients with acquired haemophilia A and treated with doses ranging from 30 to 100 U/kg during 3-26 days, inhibitors disappeared in 3 patients, persisted in one, 2 patients with inhibitors died, and 1 was removed from the study [12]. A B-domain deleted recombinant factor VIII with porcine sequence is susoctocog alfa, that was approved for the treatment of acquired hemophilia patients with severe haemorrhages [13]. Recombinant porcine FVIII (BAX801) has a cross-reactivity with human inhibitors between 30 and 50%, so it was able to reduce or stop bleeding in all 28 patients with acquired hemophilia A who received it within 24 hours. Its use can also be a solution for the haemostatic treatment of patients with congenital hemophilia with inhibitors [4].

Another B-domain-truncated molecule is also recombinant factor VIII-SingleChain, which binds to von Willebrand factor with an increased affinity [14].

Moroctocog alfa is another recombinant factor VIII product with deleted B-domain obtained from a Chinese hamster ovary cell line. A large study that included 754 patients with moderate and severe hemophilia A treated with moroctocog alfa was achieved over 20 years. The following factors predicted the plasma factor VIII level: the presence of inhibitors, the age, the body size, the race, and the analytical assay used. The weight-adjusted moroctocog alfa dose required to achieve the desired plasma level was higher in younger patients than in adolescents [15].

Obizur (OBI-1; BAX801) is a highly purified B domain-deleted recombinant swine factor VIII with similar immunogenicity and efficacy to Hyat: C (a plasma-derived factor VIII) but with a better safety profile in preclinical studies [16].

A way to improve the quality of care for hemophilic patients could be the use of a B-domain deleted FVIII which is encapsulated in lipid nanoparticles containing phosphatidylinositol. This new drug formulation was tested in inhibitory-positive mice with hemophilia A. The drug continues to be haemostatically partially effective up to 18 hours after injection, a net better result than that obtained after the administration of free B-domain deleted FVIII. The probable explanation is that the product encapsulated in lipid nanoparticles has lower binding affinity towards inhibitors, so that a larger proportion of the compound remains unbound to inhibitors [17].

### **Other less immunogenic products**

As previously noted, de novo treatment of patients with hemophilia A with recombinant factor VIII may favour inhibitors occurrence, a fact supported by some recent observational reports and a clinical trial [18]. For example, inhibitors occurred in 32.6% of patients treated with recombinant factor VIII, according to a study conducted in Saudi Arabia [19]. Researchers' concern to produce less immunogenic recombinant factor VIII products is warranted. Such a product - simoctocog alfa - was obtained from a human cell line, and it is not only efficacy and safety, but it also may to reduce the risk of inhibitors occurrence, according to some studies conducted in previously treated patients [18].

### **The fusion molecule products**

The researchers investigated the possibility of prolonging half-life of recombinant activated factor VII, which is about 2.5 hours. Thus, they made a fusion molecule between factor VII and Fc, which has a 5.5-fold extension in terminal half-life in hemophilic A mice versus recombinant activated factor VII, with comparable haemostatic properties [20].

### **The recombinant human factor II**

Recombinant human factor II is able to increase endogenous thrombin potential (ETP) in hemophiliac patients. A dose of 100 mg/L may double the ETP value even in patients with hemophilia A with inhibitors and may be a therapeutic solution for them alone or together with other bypassing agents but further studies are needed to confirm its clinical effectiveness [21].

### **The engineered activated factor V**

Another bypassing strategy consists of using an engineered factor Va variant with augmented activity. It was as effective as rh factor VIIa in terms of improving thrombin generation or lysis of the thrombus. This superfactor Va (in an amount of 200 U/kg) is comparable to rh factor

VIII regarding the correction of mean blood loss. Its combination with rh factor VIIa significantly increased the procoagulant effect [22].

#### **A zymogen-like activated factor X**

The use of a zymogen-like factor Xa with an impaired active site maturation is a possibility to maintain the activity of the prothrombinase complex. It was able to restore clot time evaluated by thromboelastometry in hemophilia A patients with and without inhibitors. This new haemostatic agent could be useful for hemophilic patients, including for those with inhibitors [23].

#### **A new immunosuppressive therapy**

Modifying antigen-specific regulatory T cells by genetic engineering using a recombinant T-cell receptor from a hemophilic patient or a factor VIII-specific chimeric antigen receptor (ANS8 CAR) may be a way to suppress the B and T lymphocyte response to the treatment with factor VIII. This seems to be a promising way to induce immune tolerance in the future [19].

#### **Other new therapeutic solutions**

The adjuvant treatment of haemorrhage in hemophilic patients with inhibitors includes the use of an aptamer (as BAX499), a product that inhibits the tissue factor pathway inhibitor [24]. A novel bypass treatment tested in animal models of hemophilia (e.g., in dogs) consists in the induction of a multi-year transgene expression of activated factor VII introduced by viral-mediated delivery [25]. Activated protein C inhibitor is another modality to compensate the factor VIII deficiency [26]. The monthly administration of recombinant tissue plasminogen activator on a central venous catheter may be a solution for the prophylaxis of device infections in hemophilic children with difficult peripheral venous access [27].

### **Conclusions**

Future therapies for hemophilic patients could consist in less immunogenic factor VIII products, new strategies that can bypass factor VIII inhibitor, the expansion of products with natural anticoagulant inhibition effect [3], the reduction of liver antithrombin production [4], or the treatment with a bispecific antibody which mimic the factor VIII function [4, 2].

### **Conflict of Interest**

The author has no conflict of interest associated with this manuscript.

### **References**

- [1] P.M. Mannucci, M.E. Mancuso, M. Franchini, Tailoring hemostatic therapies to lower inhibitor development in previously untreated patients with severe hemophilia A, *J. Thromb. Haemost.* 14 (2016)1330-1336.
- [2] J. Hartmann J, S.E. Croteau, 2017 Clinical trials update: Innovations in hemophilia therapy, *Am. J. Hematol.* 91 (2016)1252-1260.
- [3] S.L. Meeks, G. Batsuli, Hemophilia and inhibitors: current treatment options and potential new therapeutic approaches, *Hematology Am. Soc. Hematol. Educ. Program.* 2016 (2016) 657-662.
- [4] M. Shima, D. Lillicrap, R. Kruse-Jarres, Alternative therapies for the management of inhibitors, *Haemophilia.* 22(S5) (2016) 36-41.
- [5] P.E. Monahan, Emerging genetic and pharmacologic therapies for controlling hemostasis: beyond recombinant clotting factors, *Hematology Am. Soc. Hematol. Educ. Program.* 2015 (2015) 33-40.

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- [6] N. Machin, M.V. Ragni, An investigational RNAi therapeutic targeting antithrombin for the treatment of hemophilia A and B, *J. Blood Med.* 9 (2018) 135-140.
- [7] K.P. Pratt, Engineering less immunogenic and antigenic FVIII proteins, *Cell Immunol.* 301 (2016) 12-17.
- [8] G. Young, J.N. Mahlangu, Extended half-life clotting factor concentrates: results from published clinical trials, *Haemophilia.* 22(S5) (2016) 25-30.
- [9] P.M. van Helden et al., Maintenance and break of immune tolerance against human factor VIII in a new transgenic hemophilic mouse model, *Blood.* 118 (2011) 3698-3707.
- [10] S.S. Dhadwar et al., Repeated oral administration of chitosan/DNA nanoparticles delivers functional FVIII with the absence of antibodies in hemophilia A mice, *J. Thromb. Haemost.* 8 (2010) 2743-2750.
- [11] S. Mathieu S et al., Challenges of the management of severe hemophilia A with inhibitors: two case reports emphasizing the potential interest of a high-purity human Factor VIII/von Willebrand factor concentrate and individually tailored prophylaxis guided by thrombin-generation test, *Blood Coagul. Fibrinolysis.* 26 (2015) 940-945.
- [12] M.D. Tarantino et al., Recombinant porcine sequence factor VIII (rpFVIII) for acquired haemophilia A: practical clinical experience of its use in seven patients, *Haemophilia.* 23 (2017) 25-32.
- [13] C. Hart, S. Schmid, Coagulation disorders in the intensive care unit - what is new?, *Dtsch. Med. Wochenschr.* 141 (2016) 777-780.
- [14] J. Mahlangu et al., Efficacy and safety of rVIII-SingleChain: results of a phase 1/3 multicenter clinical trial in severe hemophilia A, *Blood.* 128 (2016) 630-637.
- [15] J.A. Abrantes et al., Elucidation of Factor VIII Activity Pharmacokinetics: A Pooled Population Analysis in Patients With Hemophilia A Treated With Moroctocog Alfa, *Clin. Pharmacol. Ther.* 102 (2017) 977-988.
- [16] D. Lillicrap et al., Porcine recombinant factor VIII (Obizur; OBI-1; BAX801): product characteristics and preclinical profile. *Haemophilia*, 2015. Information on: <https://www.ncbi.nlm.nih.gov/pubmed/26278557>. Accessed: 12.04.2018.
- [17] K.A. Shetty et al., Factor VIII associated with lipidic nanoparticles retains efficacy in the presence of anti-factor VIII antibodies in hemophilia A mice, *Biopharm. Drug Dispos.* 37 (2016) 409-420.
- [18] M. Franchini, P.M. Mannucci, Efficacy and safety of a recombinant factor VIII produced from a human cell line (simoctocog alfa), *Expert Opin. Drug Saf.* 16 (2017) 405-410.
- [19] J. Yoon et al., FVIII-specific human chimeric antigen receptor T-regulatory cells suppress T- and B-cell responses to FVIII, *Blood.* 129 (2017) 238-245.
- [20] J. Salas et al., Enhanced Pharmacokinetics of Factor VIIa as a Monomeric Fc Fusion, *Thromb. Res.* 135 (2015) 970-976.
- [21] K.M. Hansson et al., Effects of recombinant human prothrombin on thrombin generation in plasma from patients with hemophilia A and B, *J. Thromb. Haemost.* 13 (2015) 1293-1300.
- [22] V. Bhat et al., Improved coagulation and haemostasis in haemophilia with inhibitors by combinations of superFactor Va and Factor VIIa, *Thromb. Haemost.* 115 (2016) 551-561.
- [23] L.A. George et al., Correction of human hemophilia A whole blood abnormalities with a novel bypass agent: zymogen-like FXa(I16L), *J. Thromb. Haemost.* 13 (2015) 1694-1698.

- [24] J.Y. Chang et al., Studies on the mechanism of action of the aptamer BAX499, an inhibitor of tissue factor pathway inhibitor, *Thromb. Res.* 130(3) (2012) e151-157.
- [25] P. Margaritis. Gene-based continuous expression of FVIIa for the treatment of haemophilia, *Front. Biosci. (Schol Ed)*. 4 (2012) 287-299.
- [26] K.E. Brummel-Ziedins et al., Activated protein C inhibitor for correction of thrombin generation in hemophilia A blood and plasma, *J. Thromb. Haemost.* 9 (2011) 2262-2267.
- [27] C.E. McCarthy et al., Updated analysis: central venous access device infection rates in an expanded cohort of paediatric patients with severe haemophilia receiving prophylactic recombinant tissue plasminogen activator, *Haemophilia*. 22 (2016) 81-86.