

Past, Present and Future Options in the Treatment of Hemophilia A

ABSTRACT

Patients who present with hemophilia A require treatment to replace low levels of Factor VIII (FVIII). Intervention can either be on demand or prophylactically. The goal of therapy is to prevent joint bleeds that lead to permanent damage. The progression of therapy in hemophilia A has provided patients with options resulting in a greatly improved quality of life. The newest development of gene therapy, to replace FVIII, may be the answer to cure this disease. This review provides a historical account of the treatment for hemophilia A as well as current available treatment options.

LEARNING OUTCOMES

1. List examples of current treatments used for Hemophilia A.
2. List factors that are used to describe successful Immune Tolerance Induction
3. Describe non-factor therapy for Hemophilia A

ABBREVIATIONS: AA – aminocaproic acid, AAV – adeno associated virus, AHF – antihemophilic factor, AT – antithrombin, BHK – baby hamster kidney, BDD- B domain deleted, BU – Bethesda units, CHO - Chinese hamster ovary, DDAVP – desmopressin, EHL – extended half-life, FVIII – factor VIII, ITI – immune tolerance induction, ITRs – inverted terminal repeats, IU – International units, PEG – polyethylene glycol, PK – pharmacokinetic

response, PUPs – previously untreated patients, rFVIIa – recombinant activated factor VII, TIFP – tissue factor pathway inhibitor, TA – tranexamic acid, VWF – Von Willebrand Factor.

INDEX ITEMS: Hemophilia A, Factor VIII, Recombinant, Prophylaxis, Half-life.

INTRODUCTION

Hemophilia is one of the oldest described genetic diseases, recognized as early as the second century. Factor VIII was identified by Patek and Taylor in 1937 which they called antihemophilic factor (AHF), and an assay to identify FVIII was introduced in 1950. In 1952, hemophilia B was named Christmas disease after the first patient who was identified. When a patient's blood sample with hemophilia B was mixed with a blood sample from a patient with Hemophilia A, the results normalized, which clearly lead to the distinction that these were two separate disorders.¹

There are several ways in which bleeding episodes of hemophilia are managed. This may involve factor replacement, prophylactic treatment to prevent bleeding as well as immune tolerance induction for patients with factor inhibitors.² Ideal management of these patients should occur through a comprehensive hemophilia care center, however home administration and infusions is also common. Hemophilia patients are hospitalized when severe or life-threatening bleeds occur. The newest developments in treatments include treatment with non-factor therapy, and innovations in gene therapy.

HISTORY OF TREATMENT

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Prior to the 1900s there was no way to store blood for the treatment of hemophilia requiring that blood be transfused from one family member to another if a traumatic bleeding event occurred. The life expectancy was about 13 years of age. Early treatments included lime, bone marrow, oxygen, thyroid gland extract, hydrogen peroxide or gelatin.³ By the 1930s snake venom was used to induce blood clotting. Between 1920 and 1950 hospital-based plasma transfusions of fresh frozen plasma were the treatments utilized, however large volumes were needed to be effective. By 1960 the life expectancy in a severe hemophiliac was increased to approximately 20 years of age.³

In 1965 a plasma concentrate was discovered by Dr. Judith Pool. This concentrate known as cryoprecipitate was the product that resulted from fresh frozen plasma that had been thawed and centrifuged. This was found to be rich in factor VIII (FVIII) and could be stored and used to treat hemophiliacs undergoing surgery.³

In the 1970's lyophilized factor concentrates also became available which facilitated the treatment of joint bleeds, minimized complications and improved the quality of life. Many patients began home infusions. The life expectancy was raised to almost 60 years of age.^{4,5} These concentrates were prepared with large pools of blood donors leading to several problems including exposing patients to hepatitis A and hepatitis B. One of the most serious complications of viral contamination in the 1980's was the occurrence of human immunodeficiency virus (HIV) and AIDS causing the death of many hemophiliacs. Between 60-80% of patients were exposed to HIV.⁶ In 1992, viricidal treatment of plasma derived concentrated was implemented to eliminate exposure to these viruses. That same year recombinant FVIII was introduced following the cloning of the FVIII gene in 1984, almost a decade earlier. In 1995 preventative

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prophylaxis was being used in the treatment of hemophilia in children.⁷ Additionally, in 2000, it was shown that eliminating the use of albumin in the recombinant FVIII concentrate production process eliminated viral exposure.⁸

PRESENT DAY TREATMENT OPTIONS

The evolution of treatments in hemophilia has provided patients with several options directed at improving quality of life. Patients can be treated with plasma derived products, recombinant factors, as well as gene therapy. The newest therapy is non-factor therapy. The most challenging patients to manage are those who develop an immune response to therapy.

The goal in the management of hemophilia is to prevent bleeding and prevent the further development of arthropathy. It has been demonstrated that patients with mild/moderate hemophilia have fewer bleeding episodes resulting in delayed arthropathy. Patients with levels <3 IU of FVIII are at the highest risk, while those at 10 IU are at the lowest risk. The absence of joint bleeds occurs when FVIII levels approach 12 IU.⁹

An important consideration in the treatment of hemophilia is the individuals' pharmacokinetic responses (PK) to the therapy or concentrate they are receiving. It is important to understand that the patients' FVIII half-life is relative to their bleeding patterns in order to optimize treatment and remain cost effective.¹⁰

There are several treatment regimens that hemophiliac patients can use. The objective is to minimize joint bleeds and the damage that can occur as a result of these bleeds. Options include on demand treatment which is when factors are infused in response to a bleed. Prophylactic treatment is used to prevent bleeds by obtaining regular infusions. Frequency

of treatment depends on several factors; however it has been demonstrated that prophylaxis in children can be preventative and minimize damage from joint bleeds.¹¹

Plasma derived therapy

Fresh frozen plasma, freeze dried concentrate and cryoprecipitate work by replacing the absent or low levels of FVIII in these patients. The plasma-derived FVIII products are MonoclateP® (CSL Behring) and Hemofil-M® (Baxter). Three plasma-derived products containing both FVIII and VWF are Alphanate® (Grifols), HumateP® (CSLBehring), and Wilate ® (Octapharma). These products require frequent use and since they are plasma derived, they also need to be treated to prevent infections. Standard methods for viral inactivation is achieved by heat treatment, pasteurization, solvent/detergent treatment, monoclonal antibody separation and ultra filtration.¹² To prevent the evolution of viruses into different strains or pathogens, nucleic-acid screening and incorporating products that reduce viral activity make blood products safe from HIV and hepatitis B and C. As a result, focus has been placed on treatments that do not use human plasma.¹³ An additional complication of this type of treatment is an adverse immune reaction resulting in decreased efficacy in subsequent administration.

Previously untreated patients (PUPs) are good candidates for plasma derived products. In a study of 251 PUPs with hemophilia A it was investigated if the development of inhibitors was related to the type of concentrate used in factor replacement therapy. One hundred and twenty-five patients were assigned to receive plasma-derived FVIII-containing von Willebrand factor (vWF), and 126 were assigned to receive recombinant FVIII with no vWF. In the PUPs treated with plasma-derived products there was a 50% lower incidence of inhibitor formation.¹⁴ A

drawback of this treatment is the amount of product required to achieve hemostasis which can cause fluid overload and be problematic in pediatric patients.

Recombinant factor concentrates

Recombinant factor concentrates work by replacing FVIII levels in hemophilia patients. There have been advances in the manufacturing of coagulation proteins resulting in the development of ultra-pure recombinant concentrates derived from Chinese hamster ovary (CHO) or baby hamster kidney (BHK). The cells are transfected with human factor VII, VIII or IX gene.¹⁵

Recombinant factor concentrates have become the first line of treatment and management of acute bleeds. The first generation of products included Recombinate® (Baxter) which was derived from the full FVIII gene which contained both human albumin and animal proteins.

The product of the FVIII gene is a single polypeptide that is modified and cleaved to form light and heavy chains. Deleting the B domain of FVIII improved secretion of the cell in the recombinant process which led to the development of Refacto® which is a B domain deleted (BDD) FVIII.¹⁶ Second generation recombinant factors which include KogenateFS® (Bayer) and HelixateFS® (CSL Bering) do not use albumin in the manufacturing process and are formulated with sucrose. The third-generation recombinant factors Advate(®Baxter) and Xyntha ® (Pfizer) use no human proteins in the synthetic or final stages of production.¹⁵

Recombinant factors have been shown to be as effective as plasma derived coagulation factors. A single dose can eliminate 80% of bleeds, however the recovery time is slower than plasma derived factors.¹⁷

Inhibitor development is a major concern when using recombinant factors. This is seen in approximately 28-33% of patients. When treating patients with recombinant factors, the

body's immune system sees the derived DNA as a pathogen and tries to destroy and removes the infused FVIII.¹⁸

Extended half-life therapy (EHL)

The half-life of standard FVIII products is between 8-12 hours. Several approaches have been used to try to increase the half-life of recombinant FVIII to decrease the number of scheduled infusions.¹⁹ Recombinant FVIII is a glycoprotein bound to von Willebrand factor (vWF) which impacts the half-life of FVIII.²⁰ Activation of FVIII is induced by thrombin which in turn results in its proteolysis to an active form that is rapidly inactivated and cleared from the plasma. Several different techniques have been used to increase FVIII half-life. This includes the conjugation of FVIII with larger molecules such as polyethylene glycol(PEG), fusion to recombinant albumin, or human immunoglobulin Fc.²¹

PEGylation of FVIII extends the half-life by protecting FVII from proteolytic degradation. The albumin conjugate and the Fc fragment work by recycling the endocytosed fusion protein back into the plasma via the neonatal Fc receptor pathway. All of these processes have resulted in a modest increase in half-life of FVIII resulting in decreasing the frequency of infusions while increasing the patients' quality of life. The dependence on vWF which stabilizes FVIII is likely the reason only a modest increase in stability is achieved.²¹ The average increase in half-life is about 1.5 times compared to standard FVIII concentrates.²² It has been demonstrated in clinical studies that recombinant FVIII (rFVIII)-Fc fusion product has the longest half-life of the EHL products of approximately 19.7 hours with slightly lower rate of 14.6 hours in children 6-12 years.²²

Adjunctive Therapies for Hemophilia

There may be instances when adjunctive therapies may be used based on the type and severity of hemophilia.

Desmopressin (DDAVP) is used intravenously or intranasally in the treatment of mild or moderate hemophilia A with a response rate of 80-90%.²³ DDAVP is a vasopressin synthetic analog which causes the release of both von Willebrand factor and FVIII.²³ This can be given over the course of 2-3 days, however a decreased response occurs after the second dose as a result of tachyphylaxis.²⁴ One of the major drawbacks is its antidiuretic effect which leads to fluid retention which may cause hyponatremia and can be problematic in heart failure patients and fluid overload.²⁴

When patients present with mucosal bleeding, antifibrinolytic therapy can be used. The therapy works by preventing fibrin breakdown by inhibiting fibrinolytic enzymes found in saliva. Either aminocaproic acid (AA) or tranexamic acid (TA) can be used, however TA has a potency of ten times stronger than AA, a short half-life, and a potential for toxicity.²⁵ Administration of the drug is usually given 7 days following a surgical or dental procedure. Complications can be seen when used in hematuria and should not be given in conjunction with prothrombin complex concentrate due to the risk of thrombosis.²⁶

MANAGEMENT OF HEMOPHILIA

The goal of hemophilia treatment is to achieve a FVIII activity level sufficient to control bleeding. In patients with a mild hemorrhage, levels of 30-40 IU are sufficient. In patients with

a major hemorrhage levels should be in the range of 50 IU. In patients with life threatening hemorrhage levels should be at 100 IU¹ (Table 1).

Treatment can be administered prophylactically or on demand. Prophylaxis started in early childhood can greatly minimize joint damage as well as reduce bleeds and improve quality of life. This is the most frequent treatment used in developed countries. The goal is to maintain trough levels of $>/= 2$ IU which is achieved by the administration of FVIII three times weekly.

A dosing regimen includes the second dose to be administered 8-12 hours after the first dose. This is usually half the amount of the initial dose. When dosing patients, several factors need to be considered such as the site of bleeding, the factor half-life, as well as joint health. Determining the volume needed to achieve the required level of FVIII also depends on what type of replacement is being used to treat the patient. Recombinant FVIII concentrates are larger molecules and requires half the amount. Efficacy should be monitored by assaying plasma factor levels 15 minutes post infusion. If the target is not met, an inhibitor should be considered.¹

TREATMENT IN THE PRESENCE OF INHIBITORS

A complication of treating hemophilia is the formation of inhibitors which occurs in up to 30% of patients. The cause of the development of these neutralizing antibodies is unclear but seems to be affected by ethnicity, genetic mutation and environmental factors.²⁷ African-American hemophilia patients have twice the risk of inhibitor development compared to white hemophilia patients. This may be caused by the differences in genetic haplotypes of the patient's FVIII gene versus those found in plasma derived and recombinant products.²⁸ The use of second generation recombinant FVIII products had higher inhibitor induction rates than third generation products, which may be due to the cell line used for the

production of the product. Second generation products use BHK cells versus CHO for third generation cells. Also, the degree of product modification such as sulfation and glycosylation differ between cell types.²⁹

The extended half-life FVIII products have demonstrated a decreased immunogenicity to these new products, but requires additional studies in previously untreated patients. Some theories suggest that products prepared by Fc and albumin conjugations decrease immunogenicity by a process called antigen shielding.³⁰ This is where the fusion moiety inhibits uptake of the product by the antigen presenting cells required to produce an immune response.³¹ When PEG is used, the theory is that it inhibits the binding to the endocytosis receptors on antigen presenting cells, resulting in it being less immunogenic.³¹

The treatment of patients with inhibitors of FVIII is difficult. Bleeding episodes in patients with low-titer inhibitors (ie, concentrations below 5 Bethesda units (BU) occasionally can be overcome with high doses of FVIII. Options in other cases include a bypassing agent, recombinant activated FVII (rFVIIa). This can be used in the treatment in patients with inhibitors to prevent hemorrhagic complications.³² The normal conversion of FVII to FVIIa requires the presence of tissue factor, which in turn activates FX and FIX thus bypassing FVIII. When rFVIIa is used therapeutically requires high amount of FVIIa. Functionally some studies show that tissue factor is still required for function. Other theories suggest that FVIIa activates FX directly on the platelet surface in a tissue factor dependent manner.³³

IMMUNE TOLERANCE INDUCTION

One of the treatments for patients with inhibitors is to eradicate the inhibitors by using a process known as immune tolerance induction (ITI). This is usually performed in patients with high titer

inhibitors with Bethesda levels >5BU. This protocol involves the administration of high doses of FVIII over a period of time resulting in the adaptation of the patients' immune system to tolerate the use of the FVIII. Depending on the patient, this process can take one year or in more difficult cases up to two years.³⁴ This process is successful in 50-80% of patients.³⁵ The mechanism by which ITI works is complex and not completely understood. The immune response against FVIII occurs in two phases. The first phase involves the FVIII antigen being endocytosed, processed and presented to FVIII specific CD4+ T cells by antigen presenting cells. The second phase results in the subsequent actions between CD4+T cells and FVIII specific B cells. This allows B cell activation, cellular differentiation into plasma cells as well as antibody secretion by FVIII specific plasma cells. The development of inhibitors is dependent upon both phases while the inhibition of interactions between antigen presenting cells and T cells and/or B cells promotes antigen tolerance. The definition of tolerance is unresponsiveness to an antigen by an immune system which is fully competent which may be induced by mechanisms that involve both B and T cell interrelated modulation of immune response. These include the processes of ignorance, anergy and deletion.³⁵

Ignorance is defined as when the interaction between the FVIII antigen and the immunoglobulin and/or T cell receptors is absent. Anergy exists when an antigen encounters the lymphocyte as functionally inactivated, but it remains alive in a hyporesponsive status. This can be caused by B cells which cross link surface immunoglobulins, or by blocking signals on the surface of antigen presenting cells. Deletion is due to cell death and is obtained by hyperstimulation of both B and T cells leading to hyperexpression of the Fas surface molecule.³⁵

Successful ITI has been demonstrated in patients with FVIII BU <5, but can be seen in those with levels up to 10 BU. Also, it helps if the BU have not exceeded 200 BU and ideally stayed

below 50 BU. Starting ITI within five years of inhibitor development in a patient helps to ensure success.³⁴ Failures can be attributed to interruptions of FVIII for more than 2 weeks.

The outcome of ITI has been defined by the following criteria:³⁵

Successful ITI:

1. Undetectable inhibitor titer of <0.6 BU
2. FVIII levels recovered at 66% of the infused products.
3. Half-life of > 6 hours after a 72 hour wash out period
4. The absence of an anamnestic increase of BU after further FVIII exposure

Partial Success:

1. Reduced inhibitor titer of <5 BU/ml
2. FVIII recovery <66% of the expected values of the infused products.
3. Half-life <6 h after a 72-hour washout period
4. No inhibitor increase of > 5 BU within 6 months of on demand treatment or 12 months of being treated prophylactically.

Failure:

1. Inhibitor titer decline less than 20% over 6 months after the first 3 months of ITI
2. Failure to achieve success or partial response after 33 months of ITI.³⁵

In non-responders, a different type of factor may be tried.³⁶ However, equal response has been seen in plasma derived and recombinant factor replacement.

GENE THERAPY

Gene transfer has been successful in patients with hemophilia B, however the large size of the FVIII coding region has made successful gene therapy elusive for hemophilia A patients.

The adeno-associated virus (AAV) is a small virus from the Parvoviridae family which is made up of a non-enveloped protein shell containing a single stranded DNA. There are four non-structured rep proteins, three capsid proteins and an assembly activating protein for which the AAV genome encodes. Additionally, these are flanked by two AAV specific palindromic inverted terminal repeats (ITRs). When using these AAV vectors for gene therapy, the two ITRs are retained and the rep and cap genes are exchanged with the exogenous DNA required, in this

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case the B- domain deleted human FVIII which is then flanked by the AAV ITRs. This is known as the transgene expression cassette.³⁷

A codon-optimized adeno-associated virus serotype 5 (AAV5) vector encoding a B-domain-deleted human FVIII (AAV5-hFVIII-SQ) was injected into nine hemophilia A patients and followed for 52 weeks.³⁸ They were stratified into 3 different dosing cohorts.

The FVIII levels in the low and intermediate dose group had levels of 3 IU or less, and high dose were >5 IU.³⁸ Post gene transfer, six of seven subjects infused at a dose of 6×10^{13} vg/kg presented with levels in excess of 50 IU, which were maintained up to 1 year.³⁹

There were no inhibitors that developed, nor was immunosuppression required for this group. Bleeding rates for the group went from 16 to 1 event, and FVIII prophylactic use was stopped by week 2.³⁸

By transferring a functional gene and replacing the defective FVIII gene in a hemophilia A patient, there is a future that gene therapy may result in curing this disease.

NON-FACTOR THERAPY

An emerging treatment option for hemophilia includes therapy which doesn't rely on factor replacement or bypassing treatment. There is a new type of therapy called non-factor therapy. Several approaches have been explored including creating a new protein that works by either mimicking or replacing the activity of the factor which in this case would be FVIII or to decrease the capacity of normal clotting factor inhibitors such as antithrombin and tissue factor pathway inhibitor (TFPI),⁴⁰

The development of a humanized bispecific antibody called emicizumab is one of the most recent developments in hemophilia treatment. This non-factor therapy works by mimicking the cofactor activity of FVIII by bridging activated FIX (FIXa) and FX. By interacting with both FIXa and FX it is able to bring the enzyme (FIXa) and substrate (FX) in close proximity allowing the FIXa mediation FX activation. As demonstrated by a chromogenic assay, when purified FIXa is coupled with synthetic phospholipids, the activation of FX is enhanced by the addition of emicizumab which demonstrates this antibody can take over for some of the functions of FVIIIa.⁴¹

Emicizumab has been studied in 18 severe hemophiliacs, of which 11 had inhibitors.³⁹ Patients were given 3 different doses in which FVIII levels were expected to be 3%, 10% and 30%. Outcomes included decreased aPTTs were observed which were proportional to the expected levels of FVIII. Additionally, decreased bleeding events in all patients were observed without any formation of antibodies or thrombosis.³⁹ Emicizumab has been approved by the FDA, however it cannot be monitored by conventional FVIII assays.

Alternative strategies have been identified to address the insufficient generation of thrombin that occurs in hemophilia, in particular by reducing antithrombin levels. Fitusiran (ALN-AT3SC) works by inhibiting antithrombin (AT) and is an interfering RNA molecule. It works by

suppressing production of AT in the liver. This is in clinical trials and results have demonstrated increased thrombin generation.⁴²

Concizumab is a human monoclonal antibody that works by inhibiting tissue factor pathway inhibitor (TFPI) and enhancing thrombin generation via the tissue factor (extrinsic) pathway. In a phase 1 study which included 8 hemophilia A patients without inhibitors, treatment with concizumab demonstrated decreased bleeding episodes.⁴³ These results will ensure the continuation of phase 2 studies which will help to determine its therapeutic window.

CONCLUSION

Throughout the years, treatment for hemophilia A has progressed in leaps and bounds. There have been many options provided to patients in how and when they are treated. Both the quality and quantity of life has been greatly improved for this disease. Treatment has taken yet another turn with the implementation of non-factor therapy in patients with inhibitors.

The most impressive of all the treatments is the current success of gene therapy, which may lead to a cure for this disease. Results from patients who have undergone gene therapy have showed promise in sustaining factor levels that prevent bleeding episodes and eliminate replacement therapy. The future for hemophilia A looks promising not only for treatment options but for the possibility of curing this disease.

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Table 1: Factor levels to manage bleeds*

| Severity of Bleed | Type of bleed | Desired FVIII level | Dosing |
|--------------------------|--|----------------------------|---|
| Mild hemorrhages | Early hemarthrosis, epistaxis, gingival bleeding | 30 IU | 1-3 doses FVIII |
| Major hemorrhages | Late hemarthrosis, muscle bleeds | 50 IU | Many doses, with continuous monitoring of FVIII levels |
| Life threatening bleed | Major trauma or surgery, advanced or recurrent hemarthrosis, major GI bleeding, head trauma, compromise of limb or compartment syndrome | 80-100 IU | Continuous infusions |
| | | | |

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*The formula for dosing patients to determine the number of units of FVIII to correct the activity level is as follows:

Dose in FVIII IU = (weight in kg) x (desired FVIII increase (IU)) x (0.5 IU/kg per IU/dL)

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