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

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LEARNING OBJECTIVES

1. List examples of current treatments used for Hemophilia A.
2. List factors that are used to describe successful immune tolerance induction.
3. Describe nonfactor therapy for 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 prophylactic. 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 that result in a greatly improved quality of life. The newest development of gene therapy, a replacement for FVIII, may be the answer to cure this disorder. This review provides a historical account of the treatment of hemophilia A as well as current available treatment options.

ABBREVIATIONS: AA - aminocaproic acid, AAV - adeno-associated virus, AAV5 - adeno-associated virus serotype 5, AT - antithrombin, BHK - baby hamster kidney, BU - Bethesda units, CHO - Chinese hamster ovary, DDAVP - desmopressin, EHL - extended half-life, FIX - factor IX, FIXa - activated factor IX, VII - factor VII, FVIII - factor VIII, FX - factor X, ITI - immune tolerance induction, ITRs - inverted terminal repeats, IU - international units, PEG - polyethylene glycol, PUPs - previously untreated patients, rFVIIa - recombinant activated factor VII, rFvIII - recombinant factor VIII, TA - tranexamic acid, TFPI - tissue factor pathway inhibitor, VWF - von Willebrand factor.

INDEX TERMS: hemophilia A, factor VIII, recombinant, prophylaxis, half-life.

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INTRODUCTION

Hemophilia is one of the oldest described genetic disorders, recognized as early as the second century. Patek and Taylor first identified factor VIII (FVIII) in 1937, and they called it antihemophilic factor. An assay to identify FVIII was introduced in 1950. In 1952, hemophilia B was named Christmas disease after the first patient who was diagnosed. 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 led to the distinction that these were 2 separate disorders.[1]

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

HISTORY OF TREATMENT

Prior to the 1900s, there was no way to store blood for the treatment of hemophilia, which required that blood be transfused from 1 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 used; 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. The plasma concentrate was found to be rich in FVIII and could be stored and used to treat patients with hemophilia undergoing an operation.[3]

In the 1970s 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, which led to several problems including exposure of patients to hepatitis A and hepatitis B. One of the most serious complications of viral contamination in the 1980s was the occurrence of HIV and AIDS that caused the death of many hemophiliacs. Between 60%–80% of patients were exposed to HIV.[6] In 1992, viricidal treatment of plasma-derived concentrate was implemented to eliminate exposure to these viruses. That same year, recombinant factor VIII (rFVIII) was introduced following the cloning of the FVIII gene in 1984—almost a decade earlier. In 1995, preventive prophylaxis was being used in the treatment of hemophilia in children.[7] Additionally, in 2000, it was shown that elimination of the use of albumin in the rFVIII 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 nonfactor therapy. The most challenging patients to treat are those who develop an immune response to therapy. The goal in the treatment of hemophilia is to prevent bleeding and prevent the further development of arthropathy. It has been demonstrated that patients with mild to moderate hemophilia have fewer bleeding episodes, which results in delayed arthropathy. Patients with levels less than 3 international units (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 individual's pharmacokinetic responses to the therapy or concentrate they are receiving. It is important to understand that the patient's FVIII half-life is relative to their bleeding patterns 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 because 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 preventive and can 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 patients. The plasma-derived FVIII products are Monoclate-P® (CSL Behring) and Hemofil M (Baxter). Three plasma-derived products containing both FVIII and von Willebrand factor (VWF) are Alphanate® (Grifols), Humate-P (CSL Behring), and wilate® (Octapharma). These products require frequent use and, because they are plasma-derived, they also need to be treated to prevent infections. Standard methods for viral inactivation are achieved by heat treatment, pasteurization, solvent/detergent treatment, monoclonal antibody separation, and ultrafiltration.[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, which results 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 VWF, and 126 were assigned to receive rFVIII 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 ultrapure recombinant concentrates derived from Chinese hamster ovary (CHO) or baby hamster kidney (BHK). The cells are transfected with human factor VII (FVII), FVIII, or factor IX (FIX) 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 that 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®—a B-domain deleted FVIII.[16] Second-generation recombinant factors, which include Kogenate® FS (Bayer) and Helixate® FS (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 remove the infused FVIII.[18]

Extended Half-Life Therapy

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 rFVIII to decrease the number of scheduled infusions.[19] rFVIII is a glycoprotein bound to 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. The techniques include 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 the half-life of FVIII, which results in decreasing the frequency of infusions while increasing the patient's 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 the rFVIII-Fc fusion product has the longest half-life of the extended half-life (EHL) products of approximately 19.7 hours with a slightly lower rate of 14.6 hours in children 6–12 years of age.[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 analogue, which causes the release of both VWF and FVIII.[23] DDAVP 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 that 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 10 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 because of 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. Patients with major hemorrhage levels should be in the range of 50 IU. Patients with life-threatening hemorrhage levels should be at 100 IU[1] (Table 1).

Treatment can be administered prophylactically or on-demand. Prophylaxis started in early childhood can greatly minimize joint damage, 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 greater than or equal to 2 IU, which is achieved by the administration of FVIII 3 times weekly.

Table 1. Factor levels to manage bleeds

Severity of Bleed	Type of Bleed	Desired FVIII Level	*Dosage
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 operation, advanced or recurrent hemarthrosis, major gastrointestinal bleeding, head trauma, compromise of limb or compartment syndrome	80–100 IU	Continuous infusions

*The formula for patient dosage to determine the number of units of FVIII to correct the activity level is as follows: Dose in FVIII IU = (wt in kg) × (desired FVIII increase (IU)) × (0.5 IU/kg per IU/dL).

A dosage 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 giving patients doses, several factors need to be considered, such as the site of bleeding, the factor half-life, and 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. rFVIII concentrates are larger molecules and require half the amount. Efficacy should be monitored by assaying plasma factor levels 15 minutes postinfusion. 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 with 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 rrFVIII products had higher inhibitor induction rates than third-generation products, which may be owing to the cell line used to produce the product. Second-generation products use BHK cells; whereas, CHO is used for third-generation cells. Also, the degree of product modification, such as sulfation and glycosylation, differ among cell types.[29]

The EHL-FVIII products have demonstrated a decreased immunogenicity to these new products but do require additional studies in PUPs. 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, which results 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 factor VII (rFVIIa). rFVIIa can be used in the treatment of 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 factor X (FX) and FIX, thus bypassing FVIII. When rFVIIa is used, it therapeutically requires a 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 ITI. This is usually performed in patients with high titer inhibitors with Bethesda levels greater than 5 BU. This protocol involves the administration of high dosage of FVIII over a period of time, which results in the adaptation of the patients' immune system to tolerate the use of the FVIII. Depending on the patient this process can take 1 year or, in more difficult instances, up to 2 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 2 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, and antibody secretion by FVIII-specific plasma cells. The development of inhibitors is dependent on 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 that is fully competent, which may be induced by a mechanism that involves 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 crosslink surface immunoglobulins, or by blocking signals on the surface of antigen-presenting cells. Deletion is owing to cell death and is obtained by hyperstimulation of both B and T cells, which leads to hyperexpression of the Fas-surface molecule.[35]

Successful ITI has been demonstrated in patients with FVIII BU greater than 5 but can be seen in those with levels up to 10 BU. It helps if the BU have not exceeded 200 BU and have ideally stayed below 50 BU. Starting ITI within 5 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 criteria are (1) undetectable inhibitor titer of less than 0.6 BU, (2) FVIII levels recovered at 66% of

the infused products, (3) half-life of greater than 6 hours after a 72-hour washout period, and (4) the absence of an anamnestic increase of BU after further FVII exposure. The criteria for partial success are (1) reduced inhibitor titer of less than 5 BU/mL, (2) FVIII recovery less than 66% of the expected values of the infused products, (3) half-life less than 6 hours after a 72-hour washout period, and (4) no inhibitor increase of greater than 5 BU within 6 months of on-demand treatment or 12 months of being treated prophylactically. The criteria for ITI failure include (1) inhibitor titer decline less than 20% over 6 months after the first 3 months of ITI and (2) failure to achieve success or partial response after 33 months of ITI.[35]

In nonresponders, 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 nonenveloped protein shell containing a single stranded DNA. There are 4 nonstructured rep proteins, 3 capsid proteins, and an assembly activating protein for which the AAV genome encodes. Additionally, these are flanked by 2 AAV-specific palindromic inverted terminal repeats (ITRs). When using these AAV vectors for gene therapy, the 2 ITRs are retained and the rep and cap genes are exchanged with the exogenous DNA required. In this case the B-domain deletes 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 9 patients with hemophilia A and observed for 52 weeks. [38] They were stratified into 3 different dosage cohorts.

The FVIII levels in the low and intermediate dosage group had levels of 3 IU or less, and the high dosage group levels were greater than 5 IU.[38] After gene transfer, 6 of 7 participants 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 patient with hemophilia A, there is a future that gene therapy may result in curing this disorder.

NONFACTOR THERAPY

An emerging treatment option for hemophilia includes therapy that doesn't rely on factor replacement or bypassing treatment. There is a new type of therapy called nonfactor therapy. Several approaches have been explored, including creation of 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 (AT) 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 nonfactor therapy works by mimicking the cofactor activity of FVIII by bridging activated factor IX (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 activated FVIII.[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, including decreased activated partial thromboplastin time, were observed and 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 Federal Drug Administration; 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 AT levels. Fitusiran (ALN-AT3SC) works by inhibiting 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 TFPI and enhancing thrombin generation via the tissue factor (extrinsic) pathway. In a phase 1 study, which included 8 patients with hemophilia A 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 of 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 disorder. Treatment has taken yet another turn with the implementation of nonfactor 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 disorder. Results from patients who have undergone gene therapy have shown 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 disorder.

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