Over the past several decades, hemophilia treatment in Korea has progressed dramatically. It has become possible to prevent hemophilia complications by maintenance treatment as well as on-demand treatment with the help of the National Health Insurance program. Treatment and prevention of hemorrhage, prevention of joint complications, treatment and prevention of infectious complications have greatly improved the quality of life and life expectancy of hemophilia patients. However, the development of inhibitor is the most serious and challenging complication of clotting factor replacement therapy, although immune tolerance regimens and bypassing agents have shown some efficacy in countering this complication. The development of novel methods of therapy, including the use of extended half-life factors and gene therapy, will further improve the outcome of hemophilia patients. Administering the right drug to the right patients with the right dose at the right time will be necessary for treating the patient. Achievement of optimal therapeutic goals will require continued cooperation between patients and medical staff.

**Key Words:** Factor VIII, Hemophilia A, Recombinant factor VIII, Inhibitor

**Introduction**

Hemophilia A is a congenital bleeding disorder resulting from a deficiency of blood coagulation factor VIII. In hemophilia patients, repeated bleeding into the joints leads to synovial inflammation and, in turn, chronic joint problems. Once the cartilage is damaged, arthritis is in progress even though there is no bleeding. In addition, excessive bleeding is also observed after tooth extraction, circumcision, or muscular trauma. Other bleeding symptoms include central nervous system hemorrhage, hematuria, and nosebleeds.

The risk of such bleeding is divided into severe (less than 1% of normal coagulation factor VIII), moderate (1-5%), and mild (>5-40%), depending on the extent to which bleeding occurs voluntarily without special damage. In severe cases, which account for about 70% of all patients in Korea, spontaneous bleeding can occur.

According to the data of Korea Hemophilia Foundation, among the 2,354 hemophilic diseases associated with bleeding, 1,839 people were diagnosed with hemophilia A by the end of 2016 [1]. Among them, 72% was severe type, 17% moderate and 11% mild. Compared to the 1,142 regis-
tered by June 1999, the total number of new registrations is about 700 until the end of 2016, which means 30-50 new registration per year [2].

In the past, treatment for hemophilia was an on-demand treatment for hemorrhage, but it resulted in bleeding complications, thus reducing the quality of life and life expectancy. In the 1960s, Dr. Nielsen of Sweden already initiated preventive therapy and this method was effective, but this therapy was only recognized in the United States in 2007 and was recognized globally in 2014 [3-5]. It has indeed gone through a long journey.

Thus, prophylactic replacement therapy is now being used to prevent bleeding in advance by regularly supplementing insufficient coagulation factors. The price of a coagulation factor FVIII for use in hemophilia A is very expensive for lifetime use. For example, if a child weighing 20 kg is administered three times a week, 20 × 25 = 500 units (IU) are required per injection, about $25 per unit if the unit price is 500 won. It will be 3,000,000 won in four weeks. Fortunately, the cost of the treatment is covered by national medical insurance if it meets medical insurance standards. In Korea, the multi-disciplinary treatment became possible from the establishment of the Korea Hemophilia Foundation in 1991, and patients were systematically assisted [6,7].

**Early 1970s**

Prior to the 1970s, there was no effective treatment for hemophilia A in Korea, but FVIII-enriched lyophilized plasma, known as the ‘antihemophilic factors (AHF, Green Cross)’, began to be used since 1974 [8,9]. However, this agent was not enough to cover hemophiliacs and at most was only used for catastrophic bleeding or emergency surgery. The mortality rate of hemophiliacs was very high, and the average life span was under 30 years of age, which was short compared with that of the general population [10,11]. In the meantime, innovative regular therapy by Nilsson and Ahlberg has been a good direction to prevent bleeding from being treated [12]. However, these plasma products were limited in their output, and were at risk of spreading a variety of virus diseases including hepatitis B and C.

**The Age of AIDS Infection and Development of Viral Inactivation Methods (1980s)**

Acquired immunodeficiency syndrome (AIDS) was reported in two people with hemophilia in 1982, which was known to be caused by human immunodeficiency virus (HIV) infection [13-15]. Fatal AIDS occurred in hemophiliacs who had received HIV-contaminated plasma. The number of people with HIV infections in the United States was estimated at 6,000 to 10,000 people, with about 2,000 in Canada and about 4,000 in France. In Italy, about 1,300 people including 150 children died after using HIV-infected plasma. Iran, which used blood imports from France, reported approximately 300 infections. Iraq had been reported about 200 HIV infections, who had lived under enormous oppression and persecution under the Hussein control. In Japan, in the 1980s, HIV infection was found in 1,800 people, or 40% of hemophiliacs, and more than 600 people died of AIDS.

In Korea, 20 out of 122 hemophilia patients receiving human plasma were found to be infected with HIV. By comparing the genes of HIV, 2 HIV-infected persons sold blood and their plasma was delivered to the hemophiliacs [16]. Even though it was unfortunate, it was very small compared to USA, Europe and Japan. Rather, HIV infection was more common in patients with hemophilia B who imported foreign products. The use of plasma from Koreans, who had fewer HIV infections at that time than in USA and Europe, was the main reason for the reduction of HIV infection.

Disaster caused by human plasma products for patients with hemophilia has led to the search for ways to inactivate HIV [17]. As a result of improvement in the antiviral treatment, hepatitis and HIV infections were no longer present, and HIV infection was controlled with antiviral drugs. Non-A non-B hepatitis was identified as hepatitis C virus in 1989 and 13.5% of Korean hemophiliacs showed hepatitis C prevalence in the 1990s, but decreased to 1.8% in 2015 [7]. This means that the viral contamination is eliminated in the factor VIII drug, and interferon and ribavirin
treatment are effective for hepatitis C patients. Although there was concern that a new variant of Creutzfeldt-Jakob disease could be transmitted from plasma as found in the UK, a method of inactivating the hepatitis C and AIDS virus has been introduced since the late 1980s, and the contamination of blood products in hemophilia patients has become virtually unproblematic [18].

In 1987, a virus inactivating process called terminal dry heating was introduced in the AHF production process. Since 1989, with virus inactivation by solvent/detergent (S/D) method, Octa-B (currently Green Eight, Green Cross), an eighth coagulation factor concentrate has been produced and used domestically [2]. However, since the S/D method is known to have no inactivating effect on hepatitis A virus or Parvovirus B19, which does not have a lipid membrane, a high purity eighth coagulation factor (Green Mono, Green Cross) has been manufactured with the use of monoclonal antibody, and is still in use [19]. Another plasma agent is monoclate-P (CSL Behring) in Korea.

Plasma preparations according to recent manufacturing methods are widely used worldwide because they are safe from hepatitis B and C, or HIV infection. However, concerns about the emergence of future possible organisms capable of infecting blood, the risk of transmission of Creutzfeldt-Jakob disease or prion variants, are not completely eroded [20].

### Gene Recombinant Products Era after 1990s

In an effort to overcome the limitations of the supply shortage of plasma products, with efforts to dissipate any form of infection, the hemophilia A treatment has developed into a recombinant drug. In 1989, a recombinant factor VIII (rFVIII) was produced and demonstrated to be effective for the first time in two patients [21]. The recombinant therapeutic agents are classified into three stages depending on the stage of using the animal/human protein. The 1st generation is a product made by using animal/human protein in all the processes of 1) cell culture, 2) purification process, and 3) final stabilization. The second generation is a product made by using animal/human protein in the processes of 1) and 2). The third generation is a product made without using animal/human proteins in all processes of 1)-3). The third generation recombinant agent, unlike previous generations, reduces the risk of virus and prion infections by minimizing contact with animal/human proteins [22].

Table 1 summarizes the therapeutic agents for recombinant hemophilia used so far. It is evolving into 2nd generations, and then into 3rd generations that do not use animal/human proteins. It is known that there is little difference in vivo recovery and half-life between the first-generation recombinant agent (Recombinate, Baxter, now

### Table 1. Drugs based on the recombinant factor VIII products according to generation

| Product generation | Brand names | Manufacturer | Producer cell line | Heterologous genes | Proteins in the culture medium | Stabilizing agent | Viral inactivation/ removal |
|--------------------|-------------|--------------|--------------------|--------------------|-------------------------------|-------------------|---------------------------|
| First              | Recombinate | Baxter       | CHO                | FVIII, vWF         | BSA, aprotinin                | HSA               | No                        |
|                    | Kogenate    | Bayer Healthcare | BHK               | FVIII              | HPP                           | HSA               | No                        |
|                    | Helixate    | Bayer Healthcare | BHK               | FVIII              | HPP                           | HSA               | No                        |
|                    | Bioclate    | Baxter        | CHO                | FVIII, vWF         | BSA, aprotinin                | HSA               | No                        |
| Second             | ReFacto     | Pfizer        | CHO                | FVIII BDD SQ       | BSA                           | Sucrose           | SD                        |
|                    | Kogenate FS | Bayer Healthcare | BHK               | FVIII              | HPP                           | Sucrose           | SD                        |
|                    | Helixate FS | Bayer Healthcare | BHK               | FVIII              | HPP                           | Sucrose           | SD                        |
| Third              | Advate      | Baxter        | CHO                | FVIII, vWF         | No                            | Mannitol, trehalose | SD                        |
|                    | Xyntha      | Pfizer        | CHO                | FVIII BDD SQ       | No                            | Sucrose           | SD, NF (35 nm)            |
|                    | ReFacto AF  | Pfizer        | CHO                | FVIII BDD SQ       | No                            | Sucrose           | SD, NF (35 nm)            |
|                    | GreenGene F | Green Cross   | CHO                | FVIIIIBDD,vWF      | No                            | Amino acid        | SD, NF (20 nm)            |

CHO, Chinese hamster ovary; HPP, human plasma protein; HSA, human serum albumin; BHK, baby hamster kidney; BSA, bovine serum albumin; BDD, B-domain deleted; SD, treatment with a solvent and a detergent; NF, nanofiltration.
Shire) and the third-generation recombinant agent (Advate, Shire) [23].

The first generation includes octocog alfa (Recombinate, Shire), octocog alfa (Kogenate, Bayer), and octocog alfa (Helixate, CSL Behring). Second generations include moroctocog alfa (ReFacto, Pfizer), octocog alfa (Kogenate-FS, Bayer), and berococag alfa (GreenGene, Green Cross). The third generation includes octocog alfa (Advate, Shire), moroctocog alfa (Xyntha, Pfizer), and berococag alfa (GreenGeneF, Green Cross). It is known that there is almost no difference between in vivo recovery and half-life between the first-generation recombinant Recombinate and the third-generation recombinant Advate. Although the first generation is good in terms of efficacy and safety, it has evolved into a second generation and a third generation in which animal/human proteins are not used as much as possible when more manufacturing methods are employed.

The first product, full-length recombinant FVIII, was made almost simultaneously by Genetics Institute and Genetech, and "octocog alfa" was called an international common name. The rFVIII, made in CHO cells, is a so-called first-generation gene recombinant produced by Recombinate and Kogenate from BHK cells. The production of full-length recombinant FVIII involves a purification step of affinity chromatography using ion exchange chromatography and monoclonal antibodies followed by a step of virus inactivation step including solvent/detergent treatment [24,25].

### B-domain Deleted (BDD) Recombinant FVIII

FVIII, which spontaneously circulates in the blood, has several types of abbreviated B domains, which are formed by protein degradation of two-chain molecules. The nature of the procoagulant of these FVIII strains was not clinically different, so a recombinant preparation with the B domain removed was made [26]. These are manufactured with the deletion of the region encoding amino acids 760-1639 (almost all B domains). The FVIII form (LA-VIII variants) thus made was similar to native FVIII from a biochemical point of view.

The recombinant coagulation factor VIII (FVIII SQ) variant with the B domain is designed to reconstitute the two-chain FVIII strain present in the blood, FVIII BDD SQ strain and albumin-free lyophilisate were obtained, and its lyophilized form was proven to be effective and safe. FVIII BDD SQ was named "morcocag alfa".

The first product to use FVIII BDD SQ was ReFacto and replaced with a safer phase using monoclonal antibodies, namely Xyntha and ReFacto AF [27]. The efficacy and safety of FVIII BDD SQ agents has been confirmed by clinical trials and has been used clinically [28].

GreenGene, developed from Green Cross, is a recombinant blood clotting factor with a molecular weight of about 170 kDa and a 1,425 amino acid structure in which the B domain is removed. The modified GreenGene F is the third generation recombinant FVIII [28-30].

### The Appearance of Inhibitors

An immune response can occur when external FVIII is supplied to supplement deficient coagulation factors. These inhibitors are antibodies that block the clotting activity against FVIII, which is the most serious complication [31]. The safety of hemophilia treatment agents is largely determined by the frequency of inhibitor development and the risk of infection. Antibody production of plasma-derived FVIII and recombinant FVIII products is a very serious problem. The production of antibodies inhibits the success rate of hemostatic treatment and greatly increases the cost of hemostatic treatment [32,33]. Antibody development usually occurs at 10-14 exposure days and has been reported to occur in 25-30% of patients with severe hemophilia A who were previously untreated patients (PUP) [34,35].

According to Franchini et al., the antibody positivity was 29% and 33% in the first generation Recombinate and Kogenate, respectively, and 12% and 30% in the second generation Kogenate FS and ReFacto, respectively, which was statistically different from 21% of plasma-derived FVIII [36]. In PUP children with severe haemophilia, antibody incidence was similar in the plasma and recombinant product groups [33,34]. However, there were reports that recombinant FVIII had a higher incidence of inhibitors than plasma preparations, and this need to be clarified in the future [37-39].
Muscular dystrophy, cystic fibrosis, thalassemia, and sickle cell disease are also single gene disorders, but they are not treated as effectively as hemophilia. The treatment of hemophilia has made tremendous progress over the decades. Effective treatments have not only reduced morbidity, but death rates have also improved significantly, and according to statistics from 1991 to 2012, the median life expectancy has increased by 65 years in Korea [40,41].

In addition, a variety of new drugs with long half-lives and easy to use are under development and will soon be available in the domestic market. Products such as rFVIII-Fc, BAX855, BAY94-9027, N8-GP, rFVIII-SC and rFVIII-huCL are in preparation [42]. Among them, already Eloctate (Elocta, UCB) and BAX855 (Adynovate, Shire) have passed the Ministry of Food and Drug Safety in Korea and will be used after price adjustment. This should be discussed in more detail at the next opportunity.

Treatment with gene delivery is a method of fundamentally treating hemophilia [43]. Patients have great hopes and hopes, and are expected to be achieved soon in the 1990s, but have not yet been commercialized and still have work to solve, FVIII levels are temporary and insufficient, and on the one hand there are side effects of host immune responses to viral vectors [43]. Gene therapy is within reach and will deliver a cure in the near future for hemophilia A and B, and the possibility of commercialization is not far away.

Conclusions

Management of hemophilia has been dramatically improved over the last several decades. Although the use of hemophilia concentrates has pose a risk of infection, these problems have largely been overcome as a result of global efforts. Since the 1990s, hemophilia management has been systematized as a national project. As a result of the development of the coagulation factor preparation against hemophilia and the ongoing management of patients, the quality of life and survival rate of hemophilia have improved overall. However, the problem of sequelae of hemarthropathy, the development of neutralizing antibodies, and the reduction in the compliance of coagulants are still a challenge. Treating the right drug to the right patients with the right dosing to the right timing would be a necessary treatment for the patient. In order to achieve optimal goals through treatment, it will involve working with patients and medical staff in cooperation.

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