Immunooaffinity Purification of Factor VIII

RALPH E. WEINSTEIN, MD
Division of Hematology/Oncology, University of Connecticut Health Center, Farmington, CT 06032

ABSTRACT

The development of factor VIII concentrates has greatly facilitated hemophilia care and has made the home care of hemophilia possible. However, factor VIII concentrate that has been produced using traditional methods contains large amounts of foreign proteins and viruses. This has resulted in the development of immunologic abnormalities in many hemophiliacs and has exposed many of these patients to blood-borne viruses such as the human immunodeficiency virus (HIV) and hepatitis viruses.

Factor VIII circulates in plasma in complex with the von Willebrand factor (vWF). Both factor VIII and vWF have been purified and monoclonal antibodies (mAb) have been generated to both of these proteins. When bound to a solid support, these mAb’s can be used to isolate selectively the proteins of interest. Recently, two separate procedures have been used in the immunooaffinity purification of factor VIII on a commercial scale. One product (Monoclate) has been prepared using a mAb to the vWF bound to a chromatography column. The other product (Hemophil M) uses immobilized mAb to the factor VIII molecule.

Factor VIII concentrate purified using either of these approaches is far more pure than traditional factor VIII concentrates. In addition, the use of both viral purification and viral inactivation procedures has greatly reduced the risk of viral contamination. Early clinical studies have demonstrated that these products are effective in treating bleeding episodes and that the risk of viral infection with HIV or hepatitis viruses is low. Factor VIII concentrate produced using mAb technology appears to be the product of choice in previously untransfused hemophiliacs. Its role in the treatment of patients who have already been infected with HIV is less clear.

Introduction

Hemophilia A is an X-linked recessive disorder in which decreased blood plasma levels of properly functioning procoagulant Factor VIII (VIII:C) lead to excessive bleeding. Early attempts at transfusion therapy were hampered by the excessive volume loads associated with transfusion with whole blood or...
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plasma. Cryoprecipitate, a factor VIII-rich, cold-insoluble precipitate that forms when plasma is slowly thawed at 4°C, was first used in 1964 to treat hemophilia and introduced the modern era of clotting factor replacement therapy. The development of factor VIII concentrates, prepared by glycine or polyethylene glycol precipitation of cryoprecipitate, allowed further advances in hemophilia care. These concentrates can be freeze-dried and easily stored and have made possible the home care of hemophilia.

However, these advances have not been made without any costs to the patients. Even though factor VIII concentrates are enriched approximately 400-fold over plasma, factor VIII is present in plasma in only trace amounts. In fact, less than one percent of the protein contained in these concentrates is actually factor VIII. Thus, hemophiliacs are exposed to a large load of foreign proteins. In addition, the raw material for every lot of factor VIII concentrate is a pool of cryoprecipitate collected from up to 20,000 donors. This has exposed patients receiving these products to the risk of blood-borne viruses, such as the hepatitis viruses and the human immunodeficiency virus (HIV).

Side Effects of Treatment with Factor VIII Concentrate

Antigenic Load

Factor VIII is actually a trace contaminant in concentrates not produced using monoclonal antibody technology, making up less than 1/10 of one of the protein. These concentrates contain large quantities of fibrinogen, fibronectin, and immunoglobulins and lesser amounts of von Willebrand factor and factor XIII. Clinically, this has resulted in anaphylactic reactions and other evidence of hypersensitivity. Furthermore, even prior to the introduction of HIV into the blood pool, multiply-transfused hemophiliacs demonstrated a variety of immunologic abnormalities. Laboratory findings include polyclonal hypergammaglobulinemia and elevated levels of immune complexes. Palpable splenomegaly has been found in 25 percent of a large series of heavily transfused patients and it has been reported that splenic enlargement can be detected in 40 percent of hemophiliacs with the use of liver-spleen imaging. Acute hemolytic anemia can be caused by isohe-magglutinins against blood group type A, B, and AB cells, and chronic immune thrombocytopenia, now frequently associated with HIV-related illnesses, has been reported in hemophiliacs before exposure to HIV. Exposure to large quantities of foreign antigens is felt to play a role in these changes, although chronic exposure to a number of viruses may also be important in their pathogenesis.

Viral Contamination

A major disadvantage of the use of factor VIII concentrates is the risk of transmission of a variety of blood-borne viruses. Indeed, recipients of clotting factor concentrates show evidence of exposure to a number of viruses, including Epstein-Barr virus (EBV), cytomegalovirus (CMV), hepatitis B virus, non-A non-B hepatitis virus, and HIV. Infectious hepatitis has long been recognized as a serious problem in multiply transfused individuals. Hepatitis B antigen or antibodies to this agent are often found in the blood of hemophiliacs despite screening of donor plasma for evidence of hepatitis B viremia. In addition, elevations of serum transaminase enzymes are common in concentrate recipients in whom serologic markers for hepatitis B are present. This is felt to be due to non-A non-B hepatitis. In fact,
recent studies\textsuperscript{10,17} indicate that the incidence of non-A non-B hepatitis after first infusion with factor VIII concentrate is over 90 percent.

Perhaps most disturbing is the risk of infection with HIV. Since the first reported cases of the acquired immune deficiency syndrome (AIDS) in patients with hemophilia A\textsuperscript{3} in 1982, it has become clear that many patients with hemophilia have become infected with HIV. Most severe hemophiliacs were exposed to HIV between 1980 and 1983, the period in which that virus was prevalent in the blood pool and before assays to detect antibody to HIV were available. It is estimated that the antibody to HIV can be found in close to 90 percent of severe hemophiliacs and a lesser number of individuals with moderate or mild hemophilia. Currently, more than 600 hemophilia patients in the United States have developed AIDS,\textsuperscript{8} and the incidence of AIDS in this group remains constant. This figure represents only 5 to 10 percent of HIV antibody-positive hemophiliacs, and it is not clear how many of this large patient group will eventually develop AIDS.

The role of other viruses, such as EBV, CMV, or herpes simplex virus, in the development of AIDS is currently the subject of intense investigation. It has been postulated that these agents may cause further immune suppression in their hosts. Expression of HIV in tissue culture systems has recently been shown to be increased by antigen-induced activation of T lymphocytes\textsuperscript{14} or by concomitant herpes simplex infection,\textsuperscript{18} suggesting that chronic viral infections may reactivate latent HIV infection. In a small study, HIV antibody-positive hemophiliacs who were seropositive for EBV or CMV exhibited a greater degree of immune suppression than did HIV-positive hemophiliacs who were seronegative for these viruses.\textsuperscript{28} However, studies correlating these changes with relative risk for the development of AIDS have not yet been done.

Heat treatment of factor VIII concentrate has been shown to decrease markedly the contamination with HIV. The combination of heat treatment of factor concentrate and screening of donor plasma for the antibody to HIV have greatly reduced the risk of acquiring HIV infection from factor VIII infusion. Furthermore, wet heat treatment or treatment with solvent detergents have been shown to reduce the risk of transmission of non-A non-B hepatitis.\textsuperscript{22,25} However, the use of factor VIII concentrate that is produced using non-monoclonal technology still exposes patients to a large load of foreign antigens and, potentially to blood-borne viruses which may not be sensitive to the presently used methods of viral inactivation.

**Immunoaffinity Purification of Factor VIII**

**Technique**

Factor VIII circulates in plasma, in complex with the von Willebrand factor (vWF), a large protein that is composed of a number of identical subunits known as multimers. Both factor VIII and vWF have been purified from human plasma, and monoclonal antibodies (mAb) have been generated to both of these proteins. These antibodies possess affinity for a single epitope of the antigen and are highly specific for that molecule. By immobilizing such an antibody to a solid matrix support, one can selectively bind the protein of interest without binding other contaminating proteins. This technique has been successfully used to produce highly purified factor VIII which is free from most of the proteins that contaminate other factor VIII concentrates.

To date, two separate procedures have been used in the immunoaffinity purifi-
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cation of factor VIII on a commercial scale. These processes are outlined in figure 1. In one process, a mAb to the vWF is used to bind the factor VIII-vWF complex. Factor VIII is then eluted by the addition of calcium chloride, leaving

PRODUCTION OF MONOCLONAL FACTOR VIII PRODUCTS

(a) Use of anti-factor VIII mAb (b) Use of anti-vWF mAb

\[ \text{Cryoprecipitate} \]

\[ \begin{align*}
\text{Euglobulin Ppt} & \quad \text{Al(OH)}_3 \text{ Ppt} \\
\text{SN} & \quad \text{SN} \\
\text{VWF-VIII:C} & \quad \text{VWF-VIII:C} \\
\text{Ethylene Glycol} & \quad \text{Calcium Chloride} \\
\text{vWF-VIII:C} & \quad \text{vWF-VIII:C} \\
\text{Ion Exchange Resin} & \quad \text{Aminohexyl Agarose} \\
\text{VIII:C} & \quad \text{VIII:C} \\
\text{Lyophilization} & \quad \text{Lyophilization} \\
\text{Final Product} & \quad \text{DRY HEAT} \\
\end{align*} \]

Figure 1: Outline of methods used in the immunoaffinity purification of factor VIII. The process used to produce Hemophil M (anti-factor VIII mAb) is shown at the left and that used to produce Monoclate (anti-vWF mAb) is shown at right.
the vWF bound to the immobilized antibody. The product is then re-chromatographed on aminohexyl agarose to remove any remaining impurities. This step should separate factor VIII from any mAb that had leached from the mAb column. The resultant product has a specific activity of 700 to 3500 units per mg and is felt to be approximately 99 percent pure.31 Albumin is then added as a stabilizer so the specific activity in the final product* is much lower.

The other process now in commercial use employs a mAb which has affinity for an epitope of the factor VIII molecule itself. Using this method, factor VIII is bound directly to the mAb column so that binding of the vWF is not necessary.4 Factor VIII which has bound to the mAb column is next eluted with 40 percent ethylene glycol and further purified by ion exchange chromatography. The specific activity of factor VIII purified in this manner† is approximately 2000 units per mg of protein, and it is also stabilized by the addition of albumin. Both preparations undergo virus inactivation treatments. The product purified using the anti-vWF mAb (Monoclate) is dry heat treated after affinity purification, and concentrate prepared with the anti-factor VIII mAb (Hemophil M) is treated with a solvent-detergent step prior to affinity chromatography.

**Characteristics of Immunoaffinity Purified Factor VIII**

Both of the immunoaffinity-purified factor VIII concentrates are largely free of contaminating proteins. Fibrinogen, fibronectin, and immunoglobulins are nearly undetectable. The possibility that some of the antibody used to purify factor VIII will leech off the column and contaminate the final product is a potential concern. However, the antibody (of murine origin) is largely separated from factor VIII by the chromatography steps that follow the immunoaffinity purification. Indeed, less than 0.1 nanogram of mouse IgG can be found per unit of factor VIII in the final products.

Excluding the albumin added as stabilizer, vWF is the major "contaminant" in both preparations. The ratio of vWF antigen to factor VIII antigen is detected by immunoradiometric assay (IRMA) or enzyme-linked immunosorbent assay (ELISA) is between 1:30 and 1:115.26 Von Willebrand factor multimer analysis of the concentrate prepared using the anti-vWF mAb (Monoclate) reveals that only the lowest molecular weight multimers are present, a pattern similar to that seen in Type II variants of von Willebrand's disease.26 Thus, these preparations are not suitable for the treatment of von Willebrand's disease.

The purification procedures described here reduce the risk of viral contamination by both viral inactivation and by viral removal. The technique of monoclonal antibody purification is a highly effective method for viral removal. For instance, at least three to four logs of virus (both enveloped and non-enveloped) were removed when cryoprecipitate spiked with these viruses was subjected to chromatography on a column of anti-factor VIII mAb (as used in the purification of Hemophil M). In addition, methods for viral inactivation are also employed. The factor VIII purified with the anti-vWF mAb (Monoclate) undergoes dry-heat treatment at 60 degrees for 30 hours. This effectively kills HIV, but has little effect on the non A-non B hepatitis virus. However, as will be noted in a later section, none of the patients receiving this type of concentrate have developed hepatitis,12 so it appears that the combination of viral reduction and viral inactivation is effective for that virus. Viral inactivation with a combination of an organic solvent and a detergent is

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* Monoclate, Armour Pharmaceuticals.
† Hemophil M, Hyland; Method M, American Red Cross.
used to treat the concentrate prepared with the anti-factor VIII mAb (Hemophil M). This technique is effective for most lipid-enveloped viruses and has been demonstrated to reduce the risk of infection with non-A and non-B hepatitis virus and HIV from non-monoclonally purified factor VIII concentrate. In summary, immunoaffinity purification of factor VIII using either the anti-vWF or the anti-factor VIII has the capability to remove greater than seven to 10 logs of viruses.

Clinical Studies

Clinical studies of both mAb-purified factor VIII concentrates have been conducted in small groups of patients with hemophilia A. The half life of the anti-vWF purified product (Monoclate) averaged 15.4 hours in seven patients and that of the concentrate made with the anti-factor VIII mAb (Hemophil M) was 14.8 hours in 11 patients. These values are comparable to the 12 to 14 hour half lives of traditional factor VIII concentrates. More importantly, excellent hemostatic results have been achieved with these products.

In previously untransfused patients, there have been no conversions to HIV antibody positivity with use of either product. In addition, none of 31 patients who used concentrate produced with the anti-vWF mAb (Monoclate) or 11 patients who used factor VIII purified with the anti-factor VIII mAb (Hemophil M) have shown evidence of non-A non-B hepatitis, although two patients had transient elevation of liver enzymes. Thus, the viral purification and inactivation steps used to make these products seems effective for HIV and non-A non-B hepatitis virus.

The use of these products in patients who have already been infected with HIV, a group that comprises the majority of severe hemophiliacs at this time, is presently being studied. It is hypothesized that the use of factor VIII concentrate that is less contaminated with foreign proteins and viruses may reduce the degree of immune suppression in this patient population and may decrease their risk for developing AIDS. A small group of seven patients who all have pre-existing antibodies to HIV have been followed for over one year after beginning to receive factor VIII replacement therapy with the concentrate produced with the anti-vWF mAb (Monoclate). The investigators have found that the absolute T4 lymphocyte counts (felt to be a predictor for the development of AIDS) have not fallen as much as in a group of patients followed at the same institution for the same period of time. In addition, the patients receiving the monoclonally-purified product showed increased reactivity to a panel of standard skin antigens. It must be emphasized that these results were derived from a very small patient group and well-controlled studies with much larger treatment groups must be performed before conclusions can be drawn regarding the utility of mAb purified factor VIII concentrate in patients who have already been infected with HIV.

Conclusions

Recently, factor VIII concentrates have been produced using monoclonal antibody technology which are much more pure than traditionally-made factor VIII concentrates. These concentrates contain much less foreign proteins, and the process contains viral purification steps as well as viral inactivation steps.

These newer concentrates appear to be effective hemostatic agents and previously untreated patients who have received these products have not shown evidence of infection with HIV or hepatitis viruses. Although several investigators have theorized that these ultrapure concentrates may lessen immune suppression in patients who have already...
been infected with HIV, studies demonstrating that the use of the mAb purified factor VIII concentrates by this treatment group will decrease the risk of developing AIDS have not yet been performed.

At this time, ultrapure factor VIII concentrates are probably the treatment factor VIII concentrates by this treatment group will decrease the risk of developing AIDS have not yet been performed. At this time, ultrapure factor VIII concentrates are probably the treatment agent of choice for previously untreated hemophiliacs who require factor VIII preparations. It is unclear whether or not use of ultrapure factor VIII concentrates is beneficial for patients already infected with HIV, and it is our recommendation that these patients be entered in ongoing clinical trials that are investigating the potential benefit of these newer preparations in this patient population.

References


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