TRANSFUSION

Volume 22 September–October 1982 Number 5

Invited Review

Plasma derivatives and viral hepatitis

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VIRAL HEPATITIS is one of the most serious adverse reactions following intravenous infusions of plasma derivatives. Hepatitis A, or infectious hepatitis, has never been transmitted by a plasma derivative presumably because the characteristically short viremia in this disease usually coincides with clinical illness in adults. By contrast, both hepatitis B and non-A, non-B hepatitis are associated with characteristically long viremias in a high percentage of infected individuals. The viremia in chronically infected individuals can last for years and often for a lifetime. The risk of hepatitis B and non-A, non-B hepatitis associated with transfusions of plasma derivatives results from the manufacture of these derivatives from large pools of human plasma. These pools may contain one or more hepatitis viruses despite attempts to identify chronic hepatitis B virus carriers by screening donors for hepatitis B surface antigen (HBsAg). The risk of transmitting viral hepatitis by plasma clotting factor derivatives is not reduced significantly during their manufacture since the conditions required to retain the activity of labile plasma proteins needed for replacement therapy in individuals with deficiencies in clotting factors do not usually inactivate viruses. Transmission of viral hepatitis by plasma derivatives was documented shortly after their first use over 30 years ago.[1] The increased demand for these derivatives, because of the more widespread use of labile clotting factor concentrates in patients other than hemophiliacs and the development of newer plasma derivatives for use in non-hemophiliacs, has made methods to remove, inactivate, or immunologically neutralize hepatitis viruses in plasma derivatives more important.

Although plasma derivatives can transmit viral hepatitis, not all derivatives carry an equal risk of doing so. Plasma derivatives can be classified as either low risk or high risk with respect to transmitting viral hepatitis.[2] Low-risk products include those whose biologic activities are not markedly altered by heating, and are therefore heated at 60°C for 10 hours after packaging in dispensing vials. Heated low-risk products include *Albumin* (Cohn Fraction V) and *Plasma Protein Fraction* (Cohn Fraction IV-4 + V). Although not heated, *Immune Globulins* (Cohn Fraction II) are also low-risk products with respect to transmitting viral hepatitis. High-risk products include *Fibrinogen* (Cohn Fraction I) which is no longer a licensed product in the United States, *Anti-Hemophilic Factor* (Factor VIII concentrate, *AHF*) and *Factor IX* (II, VII, IX, X complex).[3] Newer high-risk plasma derivatives of possible clinical use include *Anti-Thrombin III*, *Fibronectin*, *α-1 Antitrypsin*, *C-1 Inactivator*, and *Factor XIII*.

Low Risk Plasma Derivatives

The presence of hepatitis B surface antigen (HBsAg) usually indicates the presence of hepatitis B virus (HBV). In fact, the ability to identify blood, plasma, or serum containing infectious HBV depends, in large part, on detection of the excess HBsAg which usually accompanies HBV. A lack of relationship between detectable HBsAg and infectious HBV in plasma derivatives results from the differential distribution of HBsAg and HBV during cold-ethanol fractionation and the heating at 60°C for 10 hours of those plasma derivatives capable of retaining biologic activity after heating.

Albumin lots often have contained HBsAg.[2] When properly manufactured, however, Albumin has never transmitted hepatitis B although in the past it could have caused an increase in antibody to HBsAg (Anti-HBs). In an early study,[3] one part of plasma containing hepatitis virus mixed with four parts of Albumin and heated at 60°C for 10 hours did not transmit hepatitis when 10 ml was injected intravenously into five human volunteers. In another study,[4] a plasma pool previ-

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Received for publication April 22, 1982.

ously shown to transmit hepatitis was heated at 60°C for either 2 or 4 hours. Both treated plasma pools transmitted hepatitis to between 40 and 50 percent of volunteer recipients; a similar attack rate was seen with the untreated plasma pool.[4] Albumin manufactured from this same plasma pool by cold-ethanol fractionation was HBsAg positive. When untreated and inoculated in 1-ml amounts into 12 human volunteers this Albumin did not transmit hepatitis B. In 100 ml amounts, however, inoculation of this Albumin produced hepatitis B in two of five volunteers. After heating at 60°C for 10 hours, 100 ml of this same Albumin failed to produce hepatitis folowing injection into eight volunteers. It is largely on the basis of these studies that, in the United States, Albumin is heated at 60°C for 10 hours.

In the past, *Plasma Protein Fraction* (PPF) lots often contained detectable HBsAg. Between 1957 and 1975, 69 percent of PPF lots contained HBsAg.[2] Although neither detailed clinical nor experimental studies have been conducted with PPF, it would seem unlikely that this plasma derivative would transmit hepatitis B since, like Albumin, it is heated at 60°C for 10 hours after manufacturing.

Neither Albumin nor PPF have been reported to transmit non-A, non-B hepatitis, the form of viral hepatitis currently responsible for approximately 90 percent of posttransfusion hepatitis cases in the United States[5] as well as many cases following plasma derivative therapy. The susceptibility of a non-A, non-B hepatitis agent to inactivation by heating at 60°C for 10 hours has recently been documented. In this study,[6] samples of plasma that reproducibly transmitted non-A, non-B hepatitis to chimpanzees were heated at 60°C for 10 hours. When inoculated into two chimpanzees, the heated samples failed to produce hepatitis; weekly determinations of serum alanine aminotransferase activity remained within normal limits and liver biopsies obtained at 1 to 2 weeks showed no evidence of hepatitis.

Only rare *Immune Globulin* lots (0.7%) have, in the past, contained HBsAg.[2] Most Immune Globulins in fact contain anti-HBs.[7,8] This antibody is associated both with recovery from hepatitis B and with immunity to reinfection by HBV.[9] Most Immune Globulin lots manufactured in the United States today have end-point dilution titers of anti-HBs of 100 or greater when tested by radioimmunoassay (RIA).[8] Historic and scientific data confirm the safety of this product with respect to transmitting hepatitis B.[8] One study in chimpanzees[10] showed that, in rare instances, Immune Globulin can transmit hepatitis B. Transmissions would require, however, high concentrations of HBV, low titers of anti-HBs, or both, in the original plasma pool from which the Immune Globulin was manufactured.[10] These conditions are very unlikely to be present in products manufactured from plasma screened and found negative for HBsAg.

Little is known about the ability of Immune Globulin to provide passive immunity against non-A, non-B hepatitis. In the late 1970s, three double-blind, randomized studies designed to evaluate immunoprophylaxis of posttransfusion hepatitis were reported.[11] In all three studies, no difference was seen between the incidence of anicteric non-A, non-B hepatitis cases among those who received Immune Globulin and those who did not. In two studies, however, there was a significant decrease in the incidence of icteric hepatitis cases among those who received Immune Globulin. This suggested that the products had modified the disease, perhaps by passive-active immunization. Immune Globulin was also shown in these studies to decrease the progression of acute non-A, non-B hepatitis to its chronic form.[12] This evidence for the partial protective effect of Immune Globulins further justifies their classification as low-risk derivatives.

In direct contrast to reports of partially effective passive immunization against non-A, non-B hepatitis in the United States is a recent report from Eastern Europe of the transmission of non-A, non-B hepatitis to 106 women and to more than one half of their babies by the intravenous injection of 1 ml of an anti-D Immune Globulin.[13] Details of the manufacturing procedure for this Immune Globulin have not been made available by the investigators. It is known that this Immune Globulin was prepared from the plasma from a few donors who were stimulated by $Rh_o(D)$ positive erythrocytes obtained from an apparent carrier of non-A, non-B hepatitis. The donors themselves developed non-A, non-B hepatitis after donating the plasma from which the globulin was made; this probably contributed greatly to the infectivity of this product.

In the absence of tests to detect antibody to non-A, non-B agents, well-designed experiments and clinical trials cannot be carried out to evaluate the protection provided by Immune Globulin against this disease. It is reassuring that Immune Globulins manufactured by cold ethanol fractionation from plasma pools containing more than 1000 individual donations (as required by federal regulation in the United States) have not been associated with the transmission of clinically recognized non-A, non-B hepatitis.

High-Risk Plasma Derivatives

In the past, *Fibrinogen, Anti-Hemophilic Factor* (AHF) and *Factor IX* lots often contained detectable HBsAg.[2] Prior to 1972, 25 percent of AHF lots and 67 percent of Factor IX lots contained HBsAg;[2] these

percentages decreased to 3 and 2 percent respectively, in 1975. Currently, all lots of AHF and Factor IX manufactured in the United States must be free of detectable HBsAg. However, even when negative for HBsAg, in the past these products often contained infectious HBV and transmitted hepatitis B with alarming frequency to susceptible recipients.[14,15] An international study of 160 hemophilia patients treated with Factor IX indicated that 67 percent either had ongoing or prior hepatitis B infections.[16] In a recent study of 136 patients with hemophilia A, 90 percent had serologic evidence of hepatitis B infections as a result of AHF therapy;[15] this included 85 percent of 34 patients 10 years of age or younger. In a separate study[17] of 29 hemophiliacs below five years of age treated with AHF or Factor IX lots prepared entirely from plasma negative for HBsAg by radioimmunoassay, 62 percent had serologic evidence of hepatitis B infection.

The use of either AHF or Factor IX to treat non-hemophilia patients introduces an increased risk of hepatitis in these recipients. Unlike multiply-treated patients with hemophilia, such recipients usually lack humoral immunity to hepatitis virus infections. The use of these products to treat acquired clotting factor deficiencies, such as severe liver disease, neonatal hemorrhagic states, cardiac bypass surgery bleeding, or to reverse the effect of coumarin, resulted in icteric hepatitis in 24 to 67 percent of recipients.[18] The frequency with which high-risk plasma derivatives transmit hepatitis B today is not known. The continued occurrence of hepatitis B, subsequent to the requirement for HBsAg testing of plasma,[18,19] suggests that HBsAg testing alone cannot assure the absence of infectious HBV in either AHF or Factor IX.

Non-A, non-B hepatitis has been transmitted by AHF[20] and Factor IX[21]; however, the frequency with which this occurs has not been determined. In one recent study,[18] all eight cardiac surgery patients treated with these products developed non-A, non-B hepatitis compared to four of 145 controls. In the second study,[22] eight of 20 patients with either mild hemophilia or Von Willebrand's disease developed non-A, non-B hepatitis after receiving Factor VIII for the first time.

A number of investigations[23,24] have characterized the chronic viral hepatitis following the use of AHF or Factor IX in hemophilia patients. Each study documented that chronic hepatitis and chronic liver disease is a significant problem in these patients; some have confirmed their findings by histologic examination of liver biopsies. Chronic liver disease can accompany the chronic hepatitis B infections which are seen in 7 to 10 percent of treated hemophiliacs,[15,24] but the majority of the histologically confirmed liver disease among hemophiliacs appears unrelated to hepatitis B.

The chronic hepatitis in hemophiliacs shares many characteristics with chronic non-A, non-B hepatitis. It is frequently an asymptomatic acute illness with low mean peak serum aminotransferase activity and, in a high proportion of infected individuals, it progresses to a chronic hepatitis characterized by widely fluctuating serum aminotransferase levels and severe liver disease histologically. In one study,[25] 63 percent of hemophiliacs had abnormal liver biopsies; half had chronic active hepatitis or cirrhosis.

New separation procedures have made possible production of a variety of plasma derivatives for the treatment of congenital or acquired deficiencies. Most of these new plasma derivatives would be expected to pose a high risk of transmitting viral hepatitis in the absence of specific treatments to inactivate the viruses. The proposed new products include *Anti-Thrombin III* for the treatment or prevention of thrombotic diseases, *Fibronectin* to treat depressed phagocytic function associated with massive trauma, α-1 *Antitrypsin* to treat congenital deficiencies, *C-1- Inactivator* to treat angioedema resulting from a congenital deficiency of this protease inhibitor, and *Factor XIII* to treat the rare cases of congenital deficiencies of this plasma enzyme.

Virus Removal from Plasma Derivatives

The distribution of HBsAg and HBV in plasma derivatives suggests that infectious HBV may preferentially be distributed into fractions from which AHF, Factor IX and the newer presumed high-risk plasma derivatives are manufactured. This conclusion is supported by data which confirm that when HBsAg-positive plasma is fractionated, HBV as well as HBV-specific DNA polymerase are in highest concentration in fractions from which high-risk plasma derivatives are prepared.[26]

Several laboratories have attempted to develop methods to remove HBsAg and HBV from high-risk plasma derivatives. Two methods, solid-phase immunoadsorption and polyethylene glycol (PEG) precipitation, have been evaluated for Factor IX concentrates.[24] Immunoadsorption consisted of incubating Factor IX concentrates with solid-phase immunoadsorbent (Sepharose 2B) to which high-titer anti-HBs was bound. In the PEG studies, crystalline PEG (4000 MW) was added to the Factor IX, and the precipitated HBsAg was removed by centrifugation. PEG precipitation is not applicable to AHF products since it precipitates factor VIII along with HBsAg. After treatment of intentionally contaminated Factor IX lots by either method, each lot was tested for infectivity in chimpanzees or gibbons; these are among

the few nonhuman primate species susceptible to HBV infections. The results of these studies suggested that both immunoadsorption and PEG precipitation was capable of partially removing HBsAg and HBV from either plasma or Factor IX but neither method was adequate to assure total HBV removal.

Inactivation or Immunologic Neutralization of Hepatitis Viruses in Plasma Derivatives

Plasma proteins cannot routinely withstand heat capable of inactivating HBV. The heating of albumin requires stabilization with acetyltryptophanate and/or caprylate. Studies have been completed and others are currently underway to evaluate methods to stabilize clotting factors to heating as heat is capable of inactivating both HBV and the agent of non-A, non-B hepatitis. Since HBV in albumin[4] but not in whole serum[26] can be inactivated by heat at 60°C for 10 hours, it would appear that information about HBV inactivation by heat will have to come from separate studies of each derivative.

Anti-thrombin III (AT-III) in 0.5M sodium citrate has been shown to withstand heating at 60°C for 10 hours with as little as 18 percent loss of biologic activity in a rabbit model.[27] (Heparin also has been shown to stabilize AT-III). In a recent study, more than 1000 chimpanzee-infectious doses of HBV added to AT-III stabilized in 0.5M sodium citrate and heated at 60°C for 10 hours, no longer transmitted hepatitis B to a susceptible chimpanzee.[28] The inactivation of HBV by heating purified stabilized AHF in solution has also been accomplished.[28] In this study, the AHF was stabilized by dissolution in a saccharose/glycine solution and heated at 60°C for 10 hours followed by dialysis. HBV added to the pooled cryoprecipitate prior to purification of the AHF was inactivated by this process.[29]

It is reasonable to be optimistic concerning stabilization to heat of most high-risk plasma derivatives in the near future. Theoretically, heating of stabilized high-risk plasma derivatives could inactivate HBV as well as the agent of non-A, non-B hepatitis, which has also been shown to be inactivated by heating at 60°C for 10 hours.[6]

In early studies, neither β-propiolactone nor ultraviolet light alone was capable of inactivating HBV although ultraviolet light did lower or remove HBV infectivity when applied in amounts which altered plasma proteins.[30] The combination of β-propiolactone plus ultraviolet light has been reported to inactivate HBV.[31] This combination of treatments has been applied to commercial preparations of some plasma derivatives. Whether this combination can be applied effectively to more labile high-risk clotting factors and whether it can routinely inactivate hepatitis viruses has not been reported. Alteration, denaturation or inactivation of proteins and residual β-propiolactone in products are additional concerns that would have to be addressed before this combination of treatments can be applied to plasma derivatives.

A recent report[32] described the removal of HBV infectivity from Factor IX by the addition of anti-HBs. In this study more than 1000 chimpanzee-infectious doses of HBV were added to each of 30 ml (750 International units) aliquots of a pool of two Factor IX lots. Two chimpanzees received this material previously incubated with 5 ml of Hepatitis B Immune Globulin (approximate anti-HBs titer 100,000 by RIA). An additional chimpanzee received the same amount of pooled Factor IX lots to which no Hepatitis B Immune Globulin was added; all aliquots were similarly handled. In the two chimpanzees which received the "treated" material, passively transferred anti-HBs and anti-HBc of the IgG subclass were detected for varying periods, but neither chimpanzee showed evidence of HBV infection. The chimpanzee receiving the "untreated" material developed hepatitis B confirmed by the development of HBsAg, anti-HBc and anti-HBs. The addition of anti-HBs either during or after the manufacture of high-risk products may prove to be a practical approach to prevent hepatitis B. The immunologic neutralization of the non-A, non-B hepatitis agent by a similar procedure would require the identification of protective antibody, which has not yet been accomplished.

Assessment of Hepatitis B Risk of Individual Plasma Derivative Lots

Testing final product plasma derivatives for HBsAg is inadequate to identify the risk of hepatitis B.[26] Testing for both anti-HBs and HBsAg has the theoretical potential to help define the hepatitis B risk associated with a plasma derivative lot. The presence of anti-HBs in a high-risk plasma derivative lot (negative for HBsAg) strongly suggests that this product will not transmit hepatitis B. The absence of detectable anti-HBs suggests the opposite since as much as 5 percent of final product AHF is immunoglobulin. Unpublished data (Gerety and Smallwood, 1982) indicate that, in 1975, 89 percent of 553 AHF lots and 48 percent of 182 Factor IX lots had no detectable anti-HBs. In 1978, however, nearly 100 percent of AHF lots and 50 percent of Factor IX lots had detectable anti-HBs. Those Factor IX lots devoid of anti-HBs in 1978 appear to identify highly purified products containing little or no immunoglobulin. Highly purified Factor IX lots, devoid of anti-HBs, therefore, may not be high risk with respect to transmitting hepatitis B. Additional work is needed to clarify whether testing for both HBsAg and anti-HBs can provide the means

to identify the risk of a plasma derivative lot with respect to transmitting hepatitis B. Final product testing to determine the risk of non-A, non-B hepatitis is not possible at this time.

Summary

Plasma derivatives can be separated into those with either a low or a high risk of transmitting viral hepatitis. Low-risk products, with few exceptions, will remain low-risk irrespective of the plasma from which they are manufactured because they are heated at 60°C for 10 hours (Albumin, Plasma Protein Fraction) or because they contain protective antibodies (Immune Globulin). This would appear to be the case not only for hepatitis B but also for non-A, non-B hepatitis.

The risk of hepatitis B associated with plasma derivatives is reduced but not eliminated by HBsAg screening of donors. Further decreasing the risk of hepatitis B associated with AHF or Factor IX lots, as well as newer products like AT-III, α-1 antitrypsin, Fibronectin, C-1 Inactivator, and Factor XIII, may be accomplished either by the combination of stabilization and heating or by assuring that these products contain an excess of anti-HBs. For highly-purified products with little residual immunoglobulin it may be necessary to add anti-HBs. The addition of antibodies against non-A, non-B hepatitis agents when they are identified, could prevent transmission of both forms of viral hepatitis by plasma derivatives. Methods to stabilize and heat high-risk plasma derivatives to inactivate hepatitis viruses have the potential to remove both hepatitis B and non-A, non-B hepatitis infectivity.

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