Viral Infectivity of Albumin and Plasma Protein Fraction

Brian L. Erstad, Pharm.D.

Original research, reviews, and case reports discussing viral infectivity of blood- and plasma-derived products were reviewed to determine the potential viral infectivity of human serum albumin (HSA) and plasma protein fraction (PPF). Data concerning viral infectivity, viral screening and inactivation procedures, and viral outbreaks associated with blood and plasma products were extracted and evaluated for pertinence to HSA and PPF. The starting material used for fractionation, the manufacturing process, postmanufacturing handling, and immunocompetence of HSA or PPF recipients were assessed to determine risk of symptomatic viral disease after transfusion. Both HSA and PPF are manufactured with pasteurization procedures that have led to an excellent viral safety record based on 50 years of clinical use. One outbreak of hepatitis B was associated with PPF as a result of an unreliable manufacturing process that has been corrected. The pasteurization process is effective in eradicating known viral pathogens when good manufacturing practices are followed. Continued surveillance of such products is warranted for viruses not included in routine screening procedures and for those that are resistant to current inactivation methods.

(Pharmacotherapy 1996;16(6):996-1001)

OUTLINE

Factors Associated with Infectivity

Starting Material

Manufacturing Process

Postmanufacturing Handling

Host Immunocompetence

Conclusion

Eliminating transfusion-related infections of viral origin is a continuing challenge for clinicians and researchers working with plasma derivatives obtained from fractionation including factor VIII, the immune globulins, human serum albumin (HSA), and plasma protein fraction (PPF). With the exception of factor VIII products that are also available from other sources (e.g., recombinant technology), all of the components are derived from pooled plasma obtained from human donors. Reports of acute hepatitis C in patients receiving one manufacturer's intravenous immunoglobulin product renewed concerns related to the safety of all blood- and plasma-derived products, particularly with respect to virus transmission. While this outbreak undergoes further analysis, the potential for virus transmission by other presumably safe products (e.g., HSA and PPF) derived from plasma fractionation deserves consideration. The term blood-derived products in this review refers to whole blood or cellular components (e.g., erythrocytes, leukocytes, platelets); plasma-derived products refers to plasma or noncellular components (e.g., factor VIII, immune globulins, HSA, PPF). Human serum albumin and PPF are plasma derivatives that are administered primarily to increase intravascular volume associated with hemodynamic instability. Several major factors determine viral infectivity of the commercially available products. In particular, the manufacturing process is where steps to inactivate viruses are most likely to differ from those for other plasma fractionation products.

From the Department of Pharmacy Practice, College of Pharmacy, University of Arizona, Tucson, Arizona.

Address reprint requests to Brian L. Erstad, Pharm.D., Department of Pharmacy Practice, College of Pharmacy, University of Arizona, Tucson, AZ 85721.
Factors Associated with Infectivity

A computerized literature search was conducted to identify articles published between 1966 and 1995 pertaining to potential or actual virus transmission by albumin and PPF. Reference lists of retrieved articles were also reviewed. In general, four major factors were found to determine whether a patient will exhibit symptomatic viral disease after transfusion: potential infectivity of the starting material (e.g., pooled plasma), effectiveness of the manufacturing process in eliminating or inactivating viruses, postmanufacturing handling of the product, and the host's immunologic competence.

Starting Material

The importance of starting material with regard to virus transmission has been known since the first studies on fractionation were conducted in the 1940s. The large number of donors required to obtain an adequate volume of plasma for fractionation increased the probability of viral contamination. Suitable animal models and techniques for identifying virus-contaminated plasma were not available during these early investigations, so human volunteers were sometimes inoculated with source material (e.g., plasma) to test for the presence of viruses known to cause hepatitis. Source material containing the virus was then used in subsequent experiments to test the infectivity of albumin solutions. The paucity of volunteers restricted the number of experiments that could be conducted in this manner.

Albumin and PPF are typically fractionated from anticoagulated venous plasma obtained from suitable donors, although other starting material (e.g., albumin from human placenta) is acceptable according to regulatory requirements. The plasma from a large number of donors (4000-30,000 U) is pooled before fractionation, so infectious agents contained in the plasma of any particular donor would be substantially diluted. It is not known to what degree, if any, this offsets the potentially increased risk of viral contamination associated with many donors.

Appropriate screening of prospective donors is the first step in eliminating possible virus transmission to the recipients of blood- and plasma-derived products. Donor screening with subsequent testing for transmissible diseases is extensively regulated by the Food and Drug Administration (FDA). Potential donors are given written materials in conjunction with an interview to determine their suitability. Volunteers who feel compelled to donate even if they know they do not meet the appropriate criteria may contact the blood center at a later date and explain any potential or known problems related to their donation; this is known as confidential unit exclusion. The donor information is entered into a computer data base and compared with any preexisting data. The blood is always tested for the hepatitis viruses, human T lymphotropic and human immunodeficiency (HIV) viruses, and syphilis. Specific antibody testing is performed to identify most viruses, although surrogate markers such as alanine aminotransferase and antibody to hepatitis B core antigen are still required. A National Institutes of Health Consensus Panel recommended that the alanine aminotransferase testing be discontinued with the introduction of a specific hepatitis C antibody test. The panel recommended the continued inclusion of the hepatitis B core antigen test, since it may decrease the risk of hepatitis B transmission.

Voluntary blood donation was called "the first and perhaps the most important variable" in determining the safety of blood and plasma component therapy, since paid donation was associated with an increased risk of viral contamination of donated blood. This issue is of most concern in countries that have a high percentage of paid donors; most blood donations in the United States are voluntary. The ethical and clinical issues pertaining to voluntary versus paid donations are complex, particularly regarding the implications for specific component products such as HSA and PPF.

Improved screening of donors has made the blood supply in the United States "safer than ever before" and therefore the risk of transmitting viruses is stated to be "infinitesimally low." For example, the risk of HIV transmission from transfused blood has decreased and is now estimated to be approximately 1/550,000 donations. However, improved donor screening is not a justification for being complacent in the handling of blood and plasma products even when such products are processed with procedures to inactivate and remove viruses. In addition to the hepatitis virus and HIV, B19 parvovirus and cytomegalovirus are implicated as possible transfusion-related pathogens. The list of hepatitis viruses also continues to grow, and new organisms in this group will likely be discovered.

Ethical and technical problems regarding
recipient notification and product recall may arise when pathogens are discovered that may cause disease in humans, but are not routinely screened for during blood donation or tested for during or after the manufacturing process. This is particularly a problem when the transfused pathogen causes a disease that is insidious or untreatable. The dilemmas associated with recalling blood and plasma products were recently illustrated when government and industry groups discussed what should be done when donors have Creutzfeldt-Jakob disease. The issue was raised when the American Red Cross voluntarily withdrew blood and plasma derivatives possibly tainted by two donors who were found to have this disease on postmortem examination.4

Creutzfeldt-Jakob disease is a degenerative disorder of the central nervous system that appears to be caused by infectious particles called prions. An FDA Ad Hoc Advisory Committee decided that both blood and plasma derivatives from donors with the disease be withdrawn, and that recipients of the tainted products should be notified before product withdrawal.13 The committee made this decision while acknowledging that the risk of transmission was theoretical. During open hearings on the subject, concerns were expressed by a representative of the American Association of Blood Banks Transfusion Transmitted Disease Committee regarding the lack of appropriate diagnostic tests, the difficulty in communicating risks to recipients, the absence of therapeutic interventions, and the product shortages that could result if the committee's decisions were implemented.14 The FDA eventually sent a memorandum to industry regarding the committee's decisions, but it allowed for the release of quarantined products (with warning statements) if shortages occurred.15

Although Creutzfeldt-Jakob disease is not caused by viruses and HSA and PPF were never specifically discussed, the example could have widespread implications for plasma derivatives. A spokesperson for a plasma fractionators association suggested that the FDA create a class warning for plasma products that states among other things that "the risk of transmission of infectious agents cannot be totally eliminated."15 The Secretary of the Department of Health and Human Services also announced the creation of a Blood Safety Committee composed of representatives from the FDA, National Institutes of Health, and Centers for Disease Control and Prevention to ensure the safety of blood and plasma products.16

Using recombinant technology to produce albumin could eliminate the risk of virus transmission from infected donors. The gene for albumin has been cloned and production could eventually be economically feasible. One supplier of albumin is currently studying nontherapeutic users for its yeast-based recombinant albumin product, and it seems likely that the introduction of recombinant albumin for therapeutic purposes is just a matter of time.17 Until such products are available, the focus should remain on producing blood- and plasma-derived products that are free of pathogenic organisms.

Manufacturing Process

In the Cohn cold ethanol fractionation procedure, named after its developer, increasing ethanol concentrations and decreasing pH are used to separate the various fractions of plasma by a series of precipitation steps.18 Plasma protein fraction (which must contain ≥ 83% albumin but ≤ 17% globulins as a commercial product)3 is isolated near the end of fractionation, although albumin is the last component ultimately produced. In contrast to intravenous immunoglobulin preparations derived from the Cohn process that are not heat treated,19 HSA and PPF are pasteurized for 10–11 hours at 60°C after being isolated. Cold fractionation with or without20,22 additional heat treatment appears to inactivate HIV successfully.

Rapid inactivation of HIV by heat pasteurization was specifically studied in HSA and PPF solutions when it was discovered that batches of albumin in the United Kingdom were derived from plasma containing the virus.23 It was concluded that any virus surviving cold fractionation would be rapidly inactivated by pasteurization in less than 30 minutes. The authors recommended that unscreened HSA and PPF in stock could be administered safely.

Cold fractionation alone does not reliably inactivate hepatitis viruses. This was demonstrated by the previously mentioned outbreak of hepatitis C that occurred with one manufacturer's immunoglobulin product before the institution of additional virus-inactivation procedures (solvent-detergent treatment).1 It was postulated that the outbreak may have been aggravated, if not caused, by screening donors for the presence of hepatitis C antibody that led to plasma pools
depleted of antibody for complexing the virus.\textsuperscript{24} With sufficient antibody, the virus might be inactivated or at least eliminated from the immunoglobulin fraction. However, with elimination of antibody through donor screening, the active virus may have concentrated in the immunoglobulin component during fractionation.

Hepatitis viruses are resistant to chemical and physical inactivation. In early studies, blood and plasma products contained organisms capable of producing jaundice.\textsuperscript{35} In one study 10 volunteers were given infected plasma (etiologic agent unknown but previously demonstrated to produce hepatitis) heated for 4 hours at 60°C.\textsuperscript{36} Three patients developed jaundice and two others had laboratory findings consistent with hepatitis infection. This period of heat treatment is far less than the 10 hours currently used to pasteurize HSA and PPF. An investigation suggests that increasing the pasteurization time may not eliminate hepatitis B virus from serum.\textsuperscript{27} Two chimpanzees were given serum with hepatitis B virus that had been pasteurized for 10 hours at 60°C and both developed laboratory evidence of infection. There is one case report\textsuperscript{38} of a volunteer who developed anicteric hepatitis B (serum transaminase concentration elevation) after receiving serum that had been pasteurized for 10 hours at 60°C. The serum was obtained from a healthy carrier who was positive for hepatitis B surface antigen.

In contrast to these studies involving plasma or serum, in early investigations of the infectivity of heat-treated albumin (10 hrs at 60°C), volunteers\textsuperscript{39} and patients\textsuperscript{40} did not have laboratory or clinical evidence of viral hepatitis even when the starting material was known to contain the virus. Later, some investigators even suggested that HSA and PPF might be given to increase antibodies to hepatitis B.\textsuperscript{31} All but highly purified albumin solutions contained antigen apparently devoid of infectivity yet were capable of eliciting an antibody response.\textsuperscript{32, 33} Although the studies conducted with plasma and serum raise concerns that heating HSA and PPF for the usual 10 hours may not preclude hepatitis B transmission, particularly when contamination is heavy,\textsuperscript{34} the improved donor-screening techniques mentioned earlier make such heavy contamination unlikely.

There are no published reports of hepatitis secondary to HSA administration, but one hepatitis B outbreak associated with PPF was apparently caused by an unreliable manufacturing process.\textsuperscript{35} A problem was suspected when one hospital noted a doubling of posttransfusion cases of hepatitis B infection from one year to the next, although approximately the same number of blood and plasma products had been infused each year. Eight of the nine patients who developed hepatitis B infection during one particular month had received PPF from a single lot, which suggested that PPF was the source of the outbreak. This was confirmed when additional patients who received the implicated lot were studied and found to be infected. Fortunately, the hospital blood bank had recorded lot numbers of PPF dispensed, which allowed investigators to trace the problem to one lot purchased from one manufacturer, and the product was recalled. The manufacturer indicated that a bulk pasteurization technique that did not allow adequate mixing of the material may have resulted in inadequate heating of the product.

Of interest, no cases of hepatitis B were traced to 5% HSA that had been manufactured in a similar manner, although it was not clear if the HSA was from the same lot as the PPF. This suggests possible differences in infectivity between HSA and PPF, possibly due to additional purification of albumin that occurs during Cohn fractionation. Virus transmission due to bulk pasteurization is no longer an issue with HSA and PPF since regulations promulgated by the FDA and Department of Health and Human Services require heat pasteurization of the final container.\textsuperscript{3}

The ability of the manufacturing process to remove or inactivate other hepatitis viruses (e.g., C and D) from HSA and PPF solutions has not been evaluated.\textsuperscript{36} One study suggests that heat pasteurization may be effective in eliminating or at least inactivating B19 parvovirus from HSA solutions if contamination has occurred.\textsuperscript{37} Similar studies have not been performed with PPF, which is also manufactured using heat pasteurization. Cytomegalovirus is an example of a herpesvirus that is commonly found in blood donations, with about half of all donors testing positive for the antibody.\textsuperscript{8} Leukocytes are associated with the greatest risk of transmitting cytomegalovirus, but they rarely survive cold storage for more than 5 days (i.e., blood bank storage).\textsuperscript{38} Published data are not available concerning the testing of commercially available HSA or PPF solutions for herpesviruses.

Postmanufacturing Handling

Viral contamination of HSA and PPF from
improper handling after manufacture has not been reported, although it is unlikely that isolated cases would be noticed without intensive surveillance. The products do not contain preservatives and are susceptible to microbial growth, as demonstrated by episodes of bacteremia in patients who received HSA contaminated during the manufacturing process or in the hospital setting. Although the conditions necessary for viral and bacterial growth may differ, general principles of aseptic technique must be adhered to when handling these parenteral products. Special precautions do not appear to be necessary when handling HSA and PPF.

Host Immunocompetence

Assuming a virus remains active despite the manufacturing process, expression of disease will depend on the pathogenicity of the organism and the host's immunocompetence. The latter is an increasingly important consideration with the availability of potent immunosuppressive drugs being given for a variety of medical and surgical procedures (e.g., transplantation) and disorders. These immunosuppressive agents predispose patients to a variety of infectious complications. Despite new antiviral therapies such as monoclonal antibodies to viral proteins and immunostimulatory drugs that are under investigation, the primary focus should be on preventing infection.

Transfusions, particularly with white blood cells and white cell-laden components (e.g., whole blood), have immunomodulatory effects, and at least indirect evidence suggests that plasma derivatives may cause immunomodulation independent of the white cells. In addition to the possibility of virus-contaminated product causing disease, blood and plasma transfusions have immunomodulatory effects in patients undergoing transplantation and surgery for cancer. For example, the use of whole blood in renal transplantation is associated with prolonged allograft survival. Similarly, perioperative infusions of fresh-frozen plasma were associated with increased 5-year survival in patients with colorectal cancer. The immunomodulatory effects of HSA and PPF, if any, have yet to be determined.

Conclusions

Overall, HSA and PPF have excellent safety records during several decades of use. Whereas the outbreak of hepatitis C associated with one manufacturer's intravenous immunoglobulin emphasizes the need for continued vigilance when administering blood- and plasma-derived products, it should not lead to an inappropriate indictment of HSA or PPF. These solutions undergo a heat-pasteurization process that results in an excellent viral safety record, particularly when compared with other plasma derivatives. The clinician should not become complacent when ordering these products, however, since the ability of heat pasteurization to inactivate some pathogenic viruses such as hepatitis C and D has not been tested. As with all blood and plasma products, unsubstantiated uses of HSA and PPF should be discouraged. It is also important that personnel handling and dispensing these products follow strict aseptic technique.

Although maintaining complete distribution records, including lot numbers, of HSA and PPF is not necessarily beneficial from a pharmacoeconomic standpoint, it is worth repeating that the one outbreak of hepatitis B associated with a contaminated PPF product was identified by such surveillance. Suspected product contamination and viral outbreaks should be investigated and reported to the manufacturer and FDA through the voluntary MedWatch reporting program.

Acknowledgment

The author would like to thank Glen Erstad for his support in the creation of this article.

References