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Viral safety of plasma-derived and recombinant products used in the management of haemophilia A and B

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Viral infections are a recognized complication of replacement therapy with blood products in patients with haemophilia A and B. Table 1 lists the main bloodborne viruses, with their genomic and physico-chemical characteristics. The risk of viral transmission is particularly high for coagulation factor concentrates made from large pools of plasma (from as many as 5000 or more donations). Before the introduction of virucidal methods, practically all patients with haemophilia became infected with the hepatitis viruses; since the early 1980s a large proportion of them have also become infected with the human immunodeficiency virus type 1 [1–3]. On the other hand, the risk of bloodborne infections is not negligible even for single-donor products such as fresh-frozen plasma and cryoprecipitate. Selection of low-risk donors and screening of blood donations are important measures to minimize the risk and should be implemented and continuously improved. However, these measures are not sufficient to abolish viral transmission, because a few infected donations still escape the screening process. Even though the chromatographic procedures currently used to produce high-purity coagulation factor concentrates increase safety by removing mechanically some viral burden, they are also not sufficient to abolish transmission. With the impetus provided particularly by the advent of the AIDS epidemic, in the last 10–15 years manufacturers of blood products have developed virucidal methods compatible with good yields and little loss of the biological activity of such labile proteins as coagulation factors. In most countries the use of virucidal methods is now obligatory for licensed factor VIII and IX concentrates. Methods currently in use are based upon terminal heating of the lyophilized products at 80°C ('dry-heating'); heating in solution at 60°C in the presence of stabilizers (pasteurization), in a suspension containing the organic solvent n-heptane or with hot vapour under high pressure, or adding a solvent/detergent mixture during the manufacturing process (Table 2).

In this review the efficacy of virucidal methods in preventing the occurrence of bloodborne infections will be evaluated. Evaluation of efficacy will be primarily based on the published results of prospective clinical trials carried out in haemophilic patients who had no previous exposure to any blood product before the infusion of a concentrate. These patients are the epitome of those at high risk of developing bloodborne viral infections and the most suitable clinical models to evaluate concentrate safety [4, 5]. Published cases of viral infections occurring in patients not enrolled in prospective studies are used as secondary criteria, because in these situations it is more difficult to establish a cause–effect relationship between concentrate infusion and viral infection. The methods developed to inactivate bloodborne viruses in single-donor blood products used in the treatment of haemophilia, such as fresh–frozen plasma and cryoprecipitate; and the issues of viral transmission, potentially associated with the recently licensed factor VIII products manufactured by recombinant DNA technology, are also discussed.

Dry heating

Virucidal methods based upon terminal heating of lyophilized concentrates at temperatures between 60°C

Table 1. Main bloodborne viruses transmitted by coagulation factor concentrates.

Virus	Genome	Lipid-enveloped	Size (nm)	Solvent/detergent resistant	Heat resistant
Human immunodeficiency virus, type 1	RNA	yes	80–100	no	no
Hepatitis A virus	RNA	no	27	yes	no
Hepatitis B virus	DNA	yes	42	no	no
Hepatitis C virus	RNA	yes	35–65	no	no
Hepatitis D virus	RNA	yes	35	no	no
B19 parvovirus	DNA	no	20	yes	yes

Table 2. Virucidal methods currently used in the manufacture of clotting factor concentrates.

Virucidal method	Main manufacturers using the method	Main product names	Available hepatitis safety prospective studies	Available HIV safety prospective studies
Dry heating, 80°C, 72 h	BPL, Cutter	8Y, 9A, Z8, Defix, Konyne 80*	Yes (refs 19–21)	No
Dry-heating, 60°C, 144 h	Baxter	Proplex T,* Autoplex T*	No	No
Heating in solution (pasteurization), 60°C, 10 h	Behringwerke, Armour	Haemate P (Humate P), Beriate P, Faktor IX HS, Monoclate P, Berinine HS*	Yes (refs 4, 23, 25)	Yes (refs 4, 22, 23, 25)
Vapour heating (60°C, 10 h, 1160 mbar)	Immuno	Kryobulin TIM 3, Bebulin TIM 3, Prothromplex, Immunate*, Immunine*	Yes (refs 42–45)	Yes (refs 42–45)
Heating in suspension (n-heptane), 60°C, 20 h	Alpha	Profilnine HT* Alphanine	Yes (refs 29, 30)	No
Solvent/detergent (TNBP and Tween 80, or Triton X-100, or cholate)	Alpha, Cutter, Baxter, American Red Cross, Biotransfusion, Biotest	Profilate SD*, Alphanine* Doate HP*, Hemophil M, AHF M*, Factor VIII and Factor IX Biotest*	Yes (refs 31–36)	Yes (refs 32–37)
Sodium thiocyanate plus ultrafiltration	Armour	Mononine*	No	No
Solvent/detergent plus dry heating, 80°C, 72 h	Novo Nordisk	Nordiate*	No	No
Solvent/detergent plus dry heating 100°C, 30 min	AIMA	Emoclot D.I.*, Aima FIX*	No	No
Solvent/detergent plus heating in solution (63°C, 10 h)	Octapharma	Octavi SDPlus	No	No

*Products indicated by asterisks have not been evaluated in the frame of prospective safety studies, although some studies are currently in progress.

and 68°C for periods of time between 24 and 72 h were developed by manufacturers and evaluated clinically in the early 1980s. Not only there was clear evidence that concentrates dry-heated at temperatures between 60°C and 68°C transmitted the hepatitis viruses with high frequency [6–11], but there were also 18 well-documented cases of transmission of the more heat-labile HIV [12–18]. Accordingly, these methods of dry-heating have been largely abandoned. Only two concentrates of the factor IX complex heated at 60°C are still commercially available (Proplex T and Autoplex T, Baxter) (Table 2). For these concentrates used in patients with haemophilia B, the time of exposure at 60°C (144 h) is much longer than the longest exposure time used for factor VIII concentrates that have transmitted HIV or hepatitis (up to 72 h) [7, 14]. Yet it remains to be demonstrated that these virucidal procedures are safe in patients with haemophilia B, because no study designed to answer this question has been carried out.

Dry-heating at higher temperature (80°C for 72 h) is being used for factor VIII and factor IX complex concentrates manufactured by BioProducts Laboratory (BPL) and the Scottish National Blood Transfusion Service. A factor IX complex concentrate based upon this virucidal method is also licensed in the United States (Konyne 80, Cutter). No case of hepatitis has been recorded in 51 previously untreated haemophiliacs infused with BPL or Scottish concentrates [19–21] (Table 3).

Pasteurization

This virucidal method is being used for several products,

Table 3. Cumulative results of hepatitis B and C safety studies carried out in previously untreated haemophilic patients infused with currently available virally-inactivated concentrates.

No. patients studied	Virucidal method	No. with hepatitis*	Confidence intervals of the hepatitis risk†
153 (refs 4, 23, 25)	Pasteurization	0/153	0–2%
50 (refs 44, 45)	Vapour heating	0/50	0–6%
51 (refs 19–21)	Dry-heating	0/38	0–6%
117 (refs 31–36)	Solvent/detergent	0/117	0–3%

*Some studies were carried out using elevations of transaminases as diagnostic criteria for non-A, non-B hepatitis, others using specific tests for antibodies to the hepatitis C virus.

†Expressed as one-sided 95% confidence intervals around the true risk of hepatitis, only for studies that resulted in no cases of hepatitis.

ranging from intermediate-purity products such as Haemate P (produced by Behringwerke and called Humate P in the USA) to ultrapure products such as Monoclate P (Armour). In Germany, pasteurized factor IX complex and coagulation factor IX preparations are manufactured by Behringwerke (Faktor IX HS and Berinine) (Table 2).

In terms of HIV transmission, the record of safety for pasteurization is excellent. In a large retrospective study of 155 German and Austrian haemophiliacs treated exclusively with a pasteurized concentrate since 1979 [22], no patient became infected with HIV over 9 years of observation, in contrast with the fact that during that period 47% of German haemophiliacs treated with non-virally inactivated products had become infected. Other data corroborating the efficacy of pasteurization in inactivating HIV are that no seroconversion occurred in previously untreated haemophilic patients enrolled in prospective hepatitis safety studies (see below) (Table 4).

In terms of hepatitis virus transmission, pasteurized factor VIII concentrates of intermediate-purity (Haemate P, Behringwerke) and higher purity (Beriate P, Behringwerke) were evaluated in patients with haemophilia A [4, 23]. No patient developed clinical or serological signs of hepatitis, although some developed serological signs of B19 parvovirus infection (IgM antibodies) [24]. A study completed in Germany in 98 previously untreated children (86 with haemophilia A and 12 with haemophilia B), treated exclusively with pasteurized concentrates and followed up for up to 10 years, showed no clinical and serological cases of hepatitis C infection [25]. Therefore the record of hepatitis safety of pasteurized concentrates is excellent, being based on the longitudinal observation of a total of 153 patients evaluated in the three aforementioned studies (Table 3) [4, 23, 25]. On the other hand, five cases of hepatitis B or C have been related to the use of pasteurized concentrates in haemophiliacs not enrolled in prospective studies [26–28]. The significance of these off-study instances of failure of pasteurization is less strong than that acquired from prospective studies. Nevertheless, they indicate that the risk of hepatitis after pasteurized concentrates is not absent.

Heating in suspension with n-heptane

This virucidal method is based upon heating the concentrate, after its suspension in the organic solvent n-heptane, at 60°C for 20 h. Two prospective studies evaluating the risk of hepatitis transmission by intermediate-purity factor VIII and IX complex concentrates manufactured by Alpha (Profilate and Profilnine HT) have detected five cases of hepatitis [29, 30]. As a result of these cases, solvent/detergent treatment has been preferred by Alpha for second-generation, high-purity factor VIII and factor IX concentrates (Profilate OSD and Alphanine-SD). A prospective study evaluating the safety of Alphanine-SD is currently in progress. However, the heat/heptane method is still being used for two products, a factor IX complex concentrate (Profilnine HT) and a high-purity factor IX concentrate (Alphanine).

Solvent/detergent

This virucidal method, based upon the addition to coagulation factor concentrates of an organic solvent, tri(n-butyl) phosphate (TNBP) and a detergent (sodium

Table 4. Commulative results of HIV safety studies carried out in anti-HIV-negative haemophilic patients infused with currently available virally-inactivated concentrates.

No. patients studied	Virucidal method	No. of seroconverters	Confidence intervals of the risk of seroconversion
210 (refs 4, 22, 23, 25)	Pasteurization	0/210	0–1.5%
81 (refs 42–45)	Vapour heating	0/81	0–3.7%
245 (refs 32–37)	Solvent/detergent	0/245	0–1.2%

*Expressed as one-sided 95% confidence intervals around the true risk of anti-HIV seroconversion.

cholate, Tween 80 or Triton X-100), inactivates large amounts of hepatitis B and C viruses and HIV *in vitro*, with little or no loss in coagulation factor activity. Most solvent and detergent is removed during the manufacturing process, so that the residual concentrations of these chemicals is too small to cause any harm even to repeatedly infused patients such as haemophiliacs. Being relatively simple, safe, and maintaining high yields of biological activity, solvent/detergent treatment has progressively become the virucidal method of choice among most concentrate manufacturers (Table 2). The record of viral safety of the concentrates treated with solvent/detergent is excellent. Several independent clinical studies have shown that no cases of hepatitis B, C and HIV infection had occurred in a large number of previously untreated haemophiliacs [31–37] (Tables 3 and 4). On the other hand, solvent/detergent treatment, highly effective in inactivating lipid-enveloping viruses, does not inactivate non-enveloped viruses. Parvovirus B19 infection developed in a number of susceptible haemophiliacs infused with a solvent/detergent concentrate [24]. More recently, an outbreak of infection with the hepatitis A virus, another non-enveloped virus, occurred in 85 haemophiliacs from Italy, Germany, Ireland and Belgium [38–41], all treated exclusively with solvent/detergent inactivated factor VIII concentrates (manufactured by Aima in Italy and Octapharma in Austria and Germany). The incapacity of solvent/detergent to inactivate non-enveloped viruses must be kept in mind, particularly in consideration of the possible existence of a non-enveloped non-A, non-B, non-C hepatitis virus resistant to lipid solvents.

Vapour heating

This virucidal method consists of exposing the moistened lyophilized concentrate to hot vapour at 60°C for 10 h at a pressure of 1190 mbar. It was devised and adopted by a European manufacturer (Immuno, Austria) for factor VIII and IX complex concentrates (Table 2). In a prospective study of the risk of transmitting of hepatitis and HIV carried out in 28 previously untransfused haemophiliacs from Italy, four patients developed clinical and/or serological evidence of hepatitis B infection [42], one being also co-infected with the hepatitis C virus [43]. However, a subsequent international multicentre study involving 28 patients with haemophilia A, 18 patients with haemophilia B and four patients with congenital factor VII deficiency, recorded no case of hepatitis (nor of HIV infection) [44, 45] (Tables 3 and 4). Whether the latter results are due to improvement in the virucidal method or, more likely, to improved donor selection and screening, remains unclear.

Remarks on concentrates viral safety

Virucidal treatments have dramatically improved the safety of coagulation factor concentrates in terms of HIV infections (Table 4). Since 1987, and the widespread adoption of the improved methods currently used, only six cases of HIV seroconversion have occurred after infusion of a factor IX complex concentrate treated with beta-propiolactone and UV light (a procedure since abandoned) [46], with a suspicion that such infections were the result of technical errors in the manufacturing process. Therefore it appears that, barring human error, the risk of HIV infection carried by virally inactivated concentrates is very small, probably being lower than that of the individual units from which they are derived (1 in 100,000–200,000).

The risk of transmission of the hepatitis viruses has been markedly lowered but not completely eliminated. The most frequently used virucidal methods (dry-heated at 80°C, pasteurization, solvent/detergent, vapour heating) are quite effective against the hepatitis B and C viruses, as can be judged by the cumulative results of prospective clinical studies (Table 3). However, hepatitis A may be rarely transmitted, and solvent/detergent is not effective in inactivating this virus. Current virucidal methods may not be effective against the thermoresistent, non-enveloped parvovirus B19. Several cases of infection have been reported after infusion of concentrates virally-inactivated with a pasteurization, vapour-heating and solvent/detergent [24]. Even a robust double-inactivation method (solvent/detergent plus dry-heating at 100°C for 10 min) [47] may not inactivate this highly resistant virus [48]. B19 causes erythema infectiosum, aplastic crises in patients with chronic haemolytic anaemias and immunodeficiency states and hydrops fetalis in pregnant women [49]. The latter complications are rare. Yet it is of concern that this virus is still transmitted by concentrates despite virucidal methods and chromatographic removal, because other pathogenic viruses with the same features may exist. There is a need, therefore, to develop new methods capable of inactivating even those viruses as resistant as the parvovirus.

Newer strategies

The aim of achieving absolute safety should not be abandoned. More and more manufacturers are using double-inactivation procedures (for instance a chemical method such as solvent/detergent and physical methods such as heating or ultrafiltration). A monoclonally purified factor IX concentrates (Mononine, Armour) uses a novel two-step method to remove or inactivate viruses, based on the use of a chemical such as sodium

thiocyanate and a physical process such as virus-retentive membrane ultrafiltration [50] (Table 2). Viral safety trials are currently in progress with this concentrate. Two independent virus-inactivation steps have been also included in the manufacturing process of several factor VIII concentrates (Nordiate, Novo Nordisk; Emoclot and Aima FIX, Aima; Octavi SDPlus, Octapharma) (Table 2).

Recently a new approach called nanofiltration has been developed [51]. This method is based upon the use of multilayered cellulose membranes with mean pore sizes of 15 nm, which should achieve steric exclusion of lipid enveloped and non-lipid enveloped viruses of different shapes and sizes (from 20 to 200 nm), including the hepatitis A virus and B19 parvovirus (Table 1). Currently this process is applicable in the preparations of concentrates of coagulation factors with molecular masses varying between 50 and 160 kD (such as factor IX), but it remains to be seen whether it is applicable to larger proteins such as factor VIII and von Willebrand factor [51]. Viral safety studies in humans are not yet available.

Finally, the availability of efficacious virucidal methods should not stop us from pursuing strategies aimed at preventing viral contamination of source plasma, in particular donor selection and screening, or to protect patients from viruses escaping the inactivation procedures, with measures such as vaccination against hepatitis A and B. Since it seems unlikely that, at least in the short term, complete absence of risk will be achieved, assurance of safety only comes from a continued high level of awareness and surveillance.

Virucidal methods for plasma and cryoprecipitate

Fresh-frozen plasma and cryoprecipitate are still used for the treatment of haemophilia, particularly but not exclusively in developing countries. Even though these products are usually made from single units of blood, the risk of transmitting bloodborne infectious agents is not negligible. Many virucidal methods cannot be applied as such to plasma and cryoprecipitate, mainly because of the high protein content of these products. Nevertheless, in the last few years there have been attempts to produce safer fresh-frozen plasma using modified virucidal methods. One approach has been that of adding solvent/detergent to pooled plasma [52]; another has been that of photoinactivation, based upon the addition to single plasma units of dyes such as methylene blue or toluidine blue, followed by exposure to visible light [53]; finally, pasteurization has also been attempted [54]. These virucidal methods appear effective in inactivating lipid-enveloping and non-enveloped model viruses added to plasma while preserving the immunological and functional integrity of plasma proteins [52–54]. However, there is as yet no study carried out in humans that demonstrates that these products do not transmit infectious bloodborne viruses.

There have been attempts to produce virally-inactivated cryoprecipitate in the Netherlands [55]. In Belgium, lyophilized cryoprecipitate heated at 60°C for 72 h has been the main product used in the treatment of haemophilic patients until 1990 [56]. The Global Blood Safety Initiative of the World Health Organization has carried out a feasibility study in Malaysia, Thailand and Sri Lanka to implement a small-scale production of small-pool cryoprecipitate, lyophilized and heated at 68°C for 24 h. It is anticipated that heating of lyophilized cryoprecipitate should at least eliminate the risk of HIV transmission, even though it is recognized that the hepatitis viruses are not inactivated by this treatment. There is certainly a need to foster the search and development of simple and inexpensive virucidal methods applicable in developing countries.

Safety of recombinant factor VIII

Recombinant factor VIII has been in use in humans for more than 7 years and is now licensed in several countries. Recombinant products should carry no risk of transmitting bloodborne viruses. There is, however, the theoretical risk of transmission of other pathogenic viruses that might be associated with the mammalian cell cultures used to produce factor VIII, the bovine albumin used in the cell culture medium, the murine monoclonal antibodies used to purify factor VIII, and the human albumin employed to stabilize factor VIII. The manufacturers claim that human and bovine albumin are virally inactivated, that hamster cells do not harbour human viruses, and that murine monoclonal antibodies are carefully checked. As an additional safety step, some manufacturers introduce virucidal methods in the production process. Long-term follow-up studies are currently ongoing and should provide additional information on the viral safety of recombinant factor VIII.

Conclusions

Plasma-derived concentrates of coagulation factor VIII, treated with the currently available virucidal methods, carry a negligible risk of transmitting HIV and a low risk of transmitting the hepatitis B and C viruses. Nevertheless, manufacturers of blood products should pursue the development of methods able to inactivate bloodborne viruses resistant to current virucidal methods (hepatitis A virus, parvovirus B19). The viral safety of factor IX concentrates for use in haemophilia B is less well established than that of factor VIII. Simple and inexpensive virucidal methods should be developed to inactivate viruses

in single-donor blood products, such as cryoprecipitate and fresh–frozen plasma, still used for haemophilia treatment particularly in developing countries. For all products (both plasma-derived and recombinant) continuing surveillance concerning viral safety is needed.

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