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Prions and blood products

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The transmission of Creutzfeldt–Jakob disease (CJD) by human pituitary-derived growth hormone has led to concerns that blood products might also provide a route for the iatrogenic transmission of CJD. A number of actions have been implemented by regulatory authorities to address such concerns, and numerous studies have been undertaken to determine whether or not there is a risk of CJD being transmitted in this manner. To date, no excess risk has been identified, leading to a growing consensus that there is little or no risk of long established forms of CJD being transmitted to recipients of blood products. This opinion does not extend to new variant CJD (vCJD) which is found predominantly in the UK and is believed to have resulted from the transmission of bovine spongiform encephalopathy (BSE) to humans. Unlike that of CJD, the prevalence of vCJD is not known. In addition, the detection of abnormal prion protein in the tonsils of vCJD-infected individuals has led to speculation that blood infectivity may be greater than in patients with CJD. A number of precautionary measures have been taken to address the possibility that vCJD may be transmissible by blood products; however, further scientific advances are needed to enable this risk to be defined. A suitable screening test is required to identify any infected blood donors, particularly where cellular blood components are being derived from populations believed to be at risk from BSE infection. Recent experimental data suggest that process operations used in the manufacture of plasma products may be capable of removing prion agents to a significant extent. However, further work is required to confirm these observations and to determine whether or not all potential vCJD infectivity would be removed by these means.

Key words: albumin; blood products; blood safety; Creutzfeldt–Jakob disease; factor VIII concentrate; factor IX concentrate; immunoglobulins; new variant Creutzfeldt–Jakob disease; plasma fractionation; prions.

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Introduction

Prion diseases are fatal neurodegenerative disorders characterized by a progressive spongiform encephalopathy and loss of brain function (1) that are believed to be caused by an abnormal isoform of a cellular glycoprotein known as the prion protein (2). Disorders of this type are also referred to as transmissible spongiform encephalopathies (TSEs), and they occur in a number of species. The most notable are scrapie in sheep, bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt–Jakob disease (CJD) in humans, with a new variant of CJD (vCJD) being identified in 1996 (3). CJD is a rare disorder, the origin of which is often not known; however, it is well established that prion diseases are transmissible with many different tissues demonstrating infectivity in experimental situations (4). In clinical practice CJD has been transmitted iatrogenically via medical instruments (5–8), electroencephalogram electrodes (9), corneal transplants (10), dura mater grafts (11–19), human pituitary-derived gonadotrophin (20–22), and human-

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pituitary derived growth hormone (17, 23–31). Concern that iatrogenic transmission of CJD might also occur via blood donated by individuals infected by growth hormone (32) led the US Food and Drug Administration (FDA), in 1987, to ban recipients of human pituitary-derived growth hormone from giving blood (33). Continuing speculation over the possibility of CJD transmission by blood products (26, 34–36) caused the FDA and other authorities to exclude donations from those perceived as representing a risk (Table 1). Despite these concerns, no evidence has been found of any association between the development of CJD and blood transfusion (37), and no excess risk of CJD has been identified in recipients of blood products (36, 57–62) nor in populations of heavily treated patients (63–65), nor in individuals known to have been exposed to blood products derived from donors who subsequently developed CJD (66, 67). This lack of evidence has led to a growing consensus that long-established forms of CJD

Table 1. Some measures taken in response to concern that transmissible spongiform encephalopathies (TSE) agents may be transmissible via human blood products.

Date	Reference	Event
1987 (Nov)	(33)	FDA* defers recipients of human pituitary-derived growth hormone from donating blood.
1991 (Dec)	(38)	CPMP† issues guidelines on the selection of animal substances for use in the manufacture of medicinal products.
1992	(39)	WHO‡ excludes recipients of pituitary hormones of human origin from donating blood.
1995	(40)	Council of Europe debarres as blood donors all individuals treated with extracts from human pituitary glands or who have a family history of CJD.
1995 (Aug)	(41)	FDA extends donor deferral to those with a family history of CJD and to recipients of dura mater. Plasma derivatives prepared by using a donation that would now be excluded must be withdrawn.
1996 (Mar)	(42)	CPMP guidance on plasma derived medicinal products adopts Council of European criteria for blood donor selection.
1996 (Dec)	(43)	FDA clarifies which donors are at risk of developing CJD.
1997 (Oct)	(44)	CPMP issues revised guidelines on the selection of animal substances for use in the manufacture of medicinal products.
1997 (Nov)	(45)	UK Department of Health initiates assessment of risk of exposure to vCJD infectivity via blood and blood products.
1998 (Jan)	(46)	Council of Europe extends donor deferral to recipients of dura mater and to those with a family history of CJD.
1998 (Feb)	(47)	CPMP recommends that batches of plasma derivatives be withdrawn if a donor is suspected or confirmed as having vCJD. Manufacturers to avoid using, as an excipient, albumin derived from countries where a number of cases of vCJD have occurred.
1998 (Feb)	(48)	UK Department of Health authorises importation of plasma for fractionation.
1998 (May)	(49)	UK Department of Health confirms that manufactured blood products should not be sourced from UK plasma.
1998 (July)	(50)	UK Department of Health decides that all blood components should be subjected to leucodepletion.
1998 (Sept)	(51)	FDA recommends that plasma derivatives manufactured from a donor who develops vCJD be withdrawn, but not where other forms of CJD are involved.
1999 (Jan)	(52)	Council of Europe extends donor deferral to recipients of corneal grafts.
1999 (Feb)	(53)	UK Department of Health completes assessment of risk of vCJD infectivity from blood and blood products, concluding that elimination of UK plasma products and leucodepletion will provide the most significant reduction in risk.
1999 (Apr)	(54)	CPMP issues revised guidance on the selection and use of animal products in the manufacture of medicinal products.
1999 (Aug)	(55)	FDA extends donor deferral to those who have spent 6 months or more in the UK between 1980 and 1996.
1999 (Nov)	(56)	FDA revises donor deferral to include recipients of bovine insulin, unless the product was not manufactured since 1980 from cattle in the UK.

*Food and Drug Administration, USA.
†Committee for Proprietary Medicinal Products, European Agency for the Evaluation of Medicinal Products.
‡World Health Organization.
CJD, Creutzfeldt–Jakob disease.

(sporadic CJD, familial CJD and Gerstmann–Sträusler–Scheinker (GSS) syndrome) pose little or no risk to recipients of blood products (68–70).

This opinion does not extend to vCJD, a new and distinctive form of human TSE (3, 71) that emerged following an epidemic of BSE in the UK (72) and which is believed to be caused by the BSE agent (73) probably having been transmitted to humans and other species via oral consumption of infected bovine products (74). Lack of knowledge concerning the extent of transmission of BSE to the human population, together with the detection of abnormal prion protein in lymphoreticular tissue from a vCJD patient (75), resulted in the safety of UK blood products being questioned (76). In response, the UK government decided to ban plasma products derived from UK blood donations (49, 77), to authorise the importation of plasma for fractionation (48), and to subject all blood components to leukofiltration (50) in order to reduce the concentration of white cells believed to be associated with TSE infectivity (78). Concern that vCJD may exist in populations resident outside the UK has resulted in the introduction of universal leukofiltration of blood components in a number of other countries (79), and all persons who have spent a total of at least 6 months in the UK between 1980 and 1996 have been excluded from donating blood in the USA (55, 56), Canada, New Zealand, Japan, and Austria. Similar considerations are underway in other countries. Meanwhile in the UK, some 700 000 litres of donor plasma are being destroyed annually and aspirations to self-sufficiency in blood products, and a comprehensive blood transfusion service based on unpaid volunteer donors have both been abandoned in face of the risk perceived from vCJD.

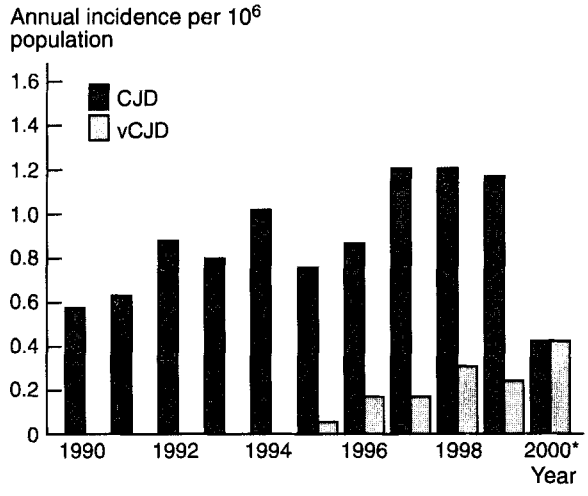

Figure 1. Incidence of deaths from Creutzfeldt–Jakob disease (CJD) and variant CJD (vCJD) in the UK between 1990 and 2000.
*Figures for the year 2000 are extrapolated from data up to 4 August, 2000. (Data are derived from (80).)

In order to determine whether or not there is a risk of vCJD being transmitted iatrogenically via blood, blood components, or plasma products, it will be necessary to determine the prevalence of presymptomatic infection in a given blood donor population, the nature of the causative agent, its concentration in infected blood or plasma donations, how it is distributed among the different components and derivatives which are obtained from blood or plasma donations, the effectiveness of blood and plasma process technologies in eliminating the causative agent, the susceptibility of individual patients to infection, the infective dose, and the nature of the dose response in clinically relevant circumstances.

Prevalence of vCJD infection

By 4 August, 2000 there were a total of 70 deaths from vCJD infection in the UK (80), with two cases in France (81) and one in the Republic of Ireland (73). This represents an annual incidence in the UK of up to 0.4 cases of vCJD per 10^6 population, compared with the expected incidence of CJD of about 1 per 10^6 population, although the number of cases of sporadic CJD reported in the UK during 2000 appears unusually low (Fig 1). By contrast, over 175 000 cases of BSE have been confirmed in cattle in the UK (73) with the possibility that significant quantities of infective material may have entered the human food chain. Some attempts have been made to predict the number of people who will develop vCJD as a consequence (74, 82, 83). Uncertainties over the degree of exposure, the susceptibility of different individuals, and the incubation period of the disease have resulted in projections ranging up to several millions, but more recent estimates have suggested that the number of cases in the UK will lie between 63 and 136 000 (84). To what extent presymptomatic vCJD may exist or occur beyond the UK is not known; however, given the extent of international travel and the significant exportation of beef products, animal feed, and livestock from the UK, it cannot necessarily be assumed that vCJD will be contained largely within the UK.

Nature of the infective agent

Although the agents responsible for TSE diseases have still to be fully defined and characterized, considerable progress has been made in determining the physicochemical nature of the infective agent. The resistance of scrapie infectivity to virucidal treatments (85, 86), a lack of any detectable association with nucleic acid (87), and the concept that a protein with self-

replicating properties may be responsible (88) led to the proposal that TSE diseases are caused by a novel proteinaceous infectious particle termed 'prion' (87). The molecular characteristics of the prion agent (PrP^{Sc}) are similar to those of a cellular glycoprotein (PrP^{C}) found in normal brain and other normal tissues (89). The principal difference between PrP^{Sc} and PrP^{C} lies in their respective conformational structures, with PrP^{Sc} exhibiting a much greater proportion of β-pleated sheet (90, 91). In addition to being associated with pathogenic behaviour (91), this particular molecular structure is responsible for a number of important properties that are characteristic of PrP^{Sc}, including resistance to proteinase-K (PK) digestion, a feature utilized in the development of immunochemical methods of detection (92, 93), a tendency to form large aggregates *in vitro* (94), and a low aqueous solubility below pH 9 (95). It is readily precipitable by ethanol (96), ammonium sulphate, and polyethylene glycol (97). PK-resistant PrP^{Sc} (denoted by PrP^{RES}) is a 27–30 kDa glycoprotein (98) that contains both hydrophobic and hydrophilic domains (99) and is normally firmly attached to cell membranes via a glycosylphosphatidylinositol (GPI) anchor (100).

Concentration of prion agents in blood

The measurement of prion agent concentration normally involves determination of the dose that causes infection in 50% of the test animals inoculated (ID_{50}). In assessing such data it is important to appreciate that the infectivity determined may be influenced by the species involved, individual genetic susceptibility, the strain of agent being studied, the route of administration, and the presence or absence of a species barrier. For example, apparent infectivity may be reduced by up to 1000-fold by a species barrier and by 10-fold by intravenous (iv) rather than intracerebral (ic) inoculation (101).

Despite the impact of the species barrier, the transmission of prion infectivity from CJD patients to rodents has been reported following ic inoculation of whole blood (102), buffy coat (103, 104), and concentrated plasma (105), results which other investigators were not able to reproduce (68, 106), leaving these findings controversial (69).

In order to circumvent the loss of sensitivity caused by the species barrier and to make more quantitative measurements, a number of investigators have established experimental rodent models by multiple passage of an exogenous prion agent such as scrapie strain 263K (107). On this basis, estimates of the concentration of hamster-adapted scrapie 263K in whole blood have ranged from 5–25 ic ID_{50} per mL (108), 10–100 ic ID_{50} per mL (109) up to 310 ic ID_{50} per mL (110), where 1 ic ID_{50} per mL is the dose given by intracerebral inoculation that results in disease in 50% of recipients. By contrast, no infectivity was transmitted in a similar model in which a mouse-

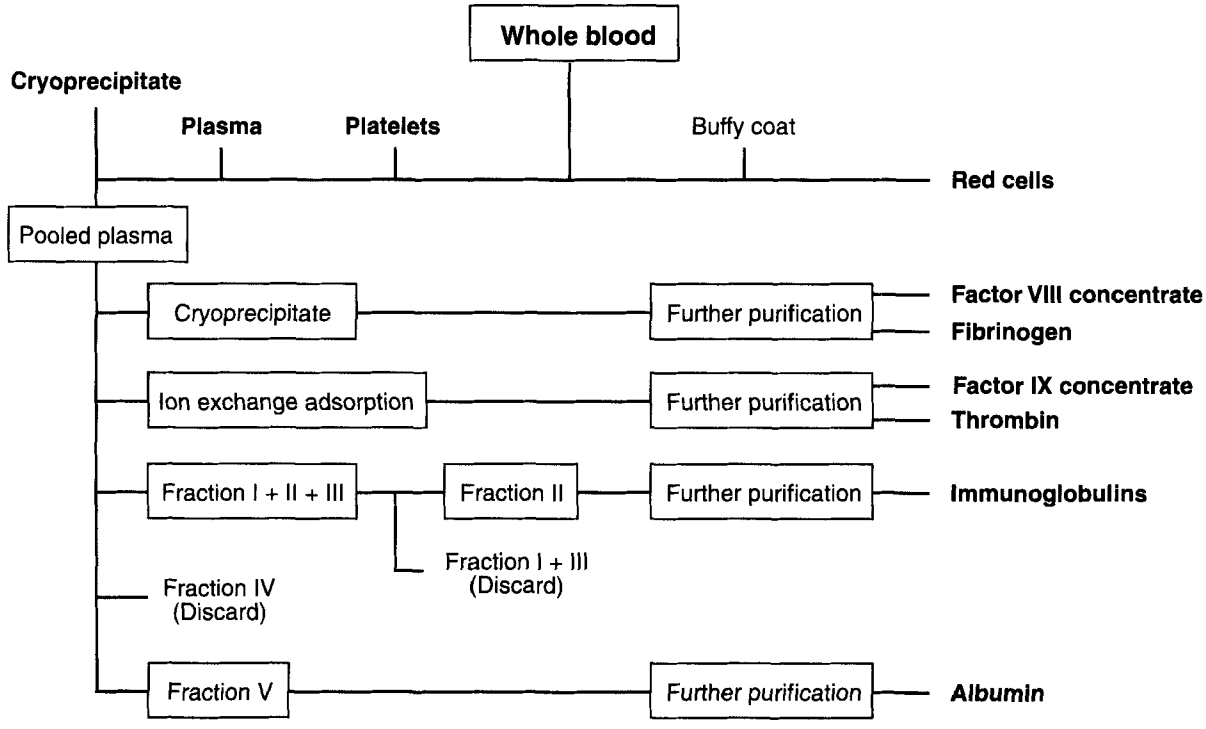

Figure 2. Flowsheet illustrating the preparation of blood components and plasma fractions from whole blood. Clinical products are indicated in bold type.

adapted strain of CJD was used (111). There are no data available concerning the concentration of the causative agent of vCJD in blood taken either from patients or from experimental animals; however, as vCJD and BSE are believed to be caused by the same prion agent (73), studies concerning BSE may be relevant to the human situation. No infectivity has been detected in bovine blood nor in any of its components either during the incubation period or in the clinical phase of BSE (112); however, given the species barrier and the limit of detection of the mouse bioassay used (113) these data can only be interpreted as below 25 000 ic ID_{50} per mL. To provide greater sensitivity, a mouse-adapted BSE (strain 301v) model has been developed, with preliminary results giving a concentration of BSE 301v in murine blood of 5 ic ID_{50} per mL (114), a level similar to that obtained in recent measurements involving hamster-adapted scrapie 263K (108). Whether or not this value is indicative of the order of vCJD infectivity in human blood remains to be determined.

Distribution of prion agents into blood components and blood products

Components and products prepared from human blood

Cellular components and proteins derived from human blood or plasma donations are in widespread clinical use (115, 116), with 90 million units of red blood cells being transfused (117) and 26 million litres of plasma processed (118) annually for this purpose worldwide. Blood components prepared from individual or a small number of pooled donations include red cell concentrates, platelets, plasma, and cryoprecipitate (Fig 2). The cellular components are separated from plasma by centrifugation, and cryoprecipitate is recovered by centrifugation following controlled thawing of frozen plasma. White cells may be separated by centrifugation and removed as a buffy coat, and their content can be reduced further by filtration (119).

Products manufactured from large volumes of pooled plasma include immunoglobulins for the treatment of immunoglobulin deficiencies, immune disorders and specific infections, albumin for volume expansion and protein replacement, factor VIII concentrate for the treatment of haemophilia A, factor IX concentrate for the treatment of haemophilia B, factor IX complex for anticoagulant reversal, and fibrinogen and thrombin for haemostasis (Fig 2). Factor VIII concentrate and fibrinogen are prepared from cryoprecipitate, usually with a number of additional purification steps, such as precipitation, adsorption, and chromatography. Factor IX concentrates are prepared by ion exchange and affinity chromatography procedures, while immunoglobulins and albumin are prepared from the intermediate precipitates fraction II and fraction V, respectively, both of which are obtained by methods of cold-ethanol (Cohn) fractionation of plasma following the removal of cryoprecipitate (120). In addition, all plasma products are subjected to procedures (not shown) that have been designed to eliminate viruses; these include solvent–detergent treatment, dry heat treatment, solution heat treatment, and acid treatment (120, 121).

Distribution of prion agents into blood components and plasma fractions

Studies concerning the distribution of prion agents into blood components and plasma fractions have been undertaken by using infected blood obtained from two different rodent models. The first studies were reported by Brown and co-workers (111, 122) who used 45 mL of blood taken from symptomatic mice infected with a strain of human CJD (the Fukuoka-1 strain of GSS syndrome) to prepare red cells, buffy coat, and plasma; 11.3 mL of plasma were then fractionated to prepare cryoprecipitate, fraction I+II+III, fraction IV_1+IV_4, and fraction V by using small-scale procedures aimed at simulating industrial manufacture. More recently, Rohwer (108, 123, 124) has presented preliminary results from a similar study with a hamster-adapted scrapie (strain 263K) model from which larger volumes of blood could be obtained for processing.

The infectivity (ic ID_{50}) of each component or fraction was determined by inoculation of a proportion of each specimen into mice (68, 111, 122) or by inoculation of all of the fraction obtained into hamsters (108, 123, 124). Some of the results from these studies are summarized in Table 2. Prion infectivity was detectable in whole blood in the hamster-adapted scrapie (108, 123, 124) and the mouse-adapted BSE (114) models as well as in buffy coat and plasma in the mouse-adapted CJD (GSS) model (111, 122). The highest infectivity was found in buffy coat, consistent with PrP^{Sc} being associated white cells (78); nevertheless, this was some five to six orders of magnitude lower than in brain extract. Small quantities of infectivity were also found in cryoprecipitate, in the first precipitate (fraction I+II+III) recovered by ethanol fractionation and in fraction V, but only when prepared from blood taken from the most highly infected animals (ie brain PrP^{Sc} titre of 10^7 ic ID_{50} per mL). No infectivity was detected in fraction II (Table 2). Different routes of administration have been assessed by using samples of buffy coat and plasma obtained from CJD (GSS)-infected mice, with iv administration giving an apparent fivefold reduction in infectivity for buffy coat and a sevenfold reduction for plasma compared with the ic route (122).

Table 2. Distribution of transmissible spongiform encephalopathy (TSE) infectivity into blood components and plasma fractions prepared from the blood of experimentally infected rodents.

Material	TSE infectivity (ic iu/mL)		
	Mouse-adapted GSS Syndrome	Hamster-adapted Scrapie 263K	Mouse-adapted BSE, 301v
(Reference)	(111, 122)	(108, 123, 124)	(114)
Brain	10^6–10^7	5×10^7	
Blood	Not detectable	4–24	5
Buffy coat	44.4–106.0		
Plasma	10.3–34.4	3.9	
Cryoprecipitate	1.2–2.6	0.6	
Fraction I+II+III	0.8	0.3	
Fraction IV	0.0–0.5		
Fraction II (IgG intermediate)		Not detectable	
Fraction V (Albumin intermediate)	0.0–0.3	Not detectable	

BSE, bovine spongiform encephalopathy; GSS, Gerstmann–Sträusler–Scheinker syndrome; ic, intracerebral inoculation; IgG, immunoglobulin G.

Although these studies are probably the best available representation of how 'natural' prion infectivity might distribute during blood processing and plasma fractionation, this experimental approach is limited by the small volumes of blood available. The relatively low titre of PrP^{Sc} in blood and plasma and the fact that PrP^{Sc} cannot be detected beyond the initial fractionation steps make it impossible to study the effect of further processes that are undertaken in the manufacture of plasma products.

Elimination of prion agents during plasma fractionation

Prion agents are highly resistant to inactivation by physical and chemical means. The mouse-adapted BSE 301v strain is particularly heat resistant, surviving autoclaving at 138 °C for 1 h (125). Therefore, elimination of prion agents in the processing of proteins and other biological products is likely to be dependent on the physical removal of the agent rather than on its inactivation by some physical or chemical treatment. Established processes utilized in the manufacture of plasma products include the separation of proteins according to differences in solubility behaviour and adsorptive behaviour. A range of technologies are used, such as precipitation, adsorption chromatography and adsorptive filtration, procedures which might be expected to lend themselves readily to the separation of PrP^{Sc} from normal plasma proteins (126).

In order to determine this capability of a process operation or a series of operations, it is necessary to challenge an accurately down-scaled model of the process with an appropriate high-titre solution of a suitable TSE agent. The degree of PrP^{Sc} reduction that is measured is usually expressed logarithmically. A number of studies of this type have been undertaken concerning plasma fractionation, and in all of these a preparation of hamster-adapted scrapie 263K was added to the starting material either as a brain homogenate (111, 127–130) or in the form of a microsomal fraction (131). The analyses were performed by bioassay (111, 127–130) or by Western blotting (127, 128, 131) by using the PrP-specific 3F4 antibody (93). Results from these investigations are summarized for processes used in the manufacture of albumin and immunoglobulin products (Table 3) and for processes used in the preparation of coagulation factor concentrates (Table 4).

Different methods of *cold ethanol fractionation* were used in these studies; Cohn–Oncley (132, 133) by Lee and co-workers (127, 128), Kistler–Nitschmann (134) by Morgenthaler (129), and modifications of Hink–Vogelaar (135, 136) by Foster and co-workers (131). Despite the different methods and experimental procedures, relatively consistent results were obtained (Table 3), suggesting that the effects of cold-ethanol fractionation on PrP^{Sc} may be fairly robust. In particular, a substantial reduction of scrapie agent was observed by precipitation into fraction III and into fraction IV, protein precipitate fractions which are discarded in the manufacture of immunoglobulin and albumin products, respectively. Results obtained by bioassay (PrP^{Sc}) and Western blotting (PrP^{RES}) were also reasonably equivalent, suggesting that *in vitro* methods of determination may be suitable for more extensive process validation studies.

Following the removal of fraction III and fraction IV by *centrifugal separation* the resultant supernatants are usually clarified by depth filtration. In addition, solutions prepared by resuspending fraction II and fraction V are also usually clarified by depth filtration.

Table 3. Reduction of Scrapie 263K by individual process steps used in the manufacture of albumin and immunoglobulins.

Product	Process step	Scrapie reduction (Log_{10})		Reference
		Bioassay	Western blot	
Albumin	Cryoprecipitation		1.0 ± 0.13	(128)
			< 1	(131)
	Fraction I precipitation		1.1 ± 0.08	(128)
	Fraction (I)+II+III precipitation	2.1		(111)
		2.1		(129)
		4–5	4–5	(127)
			1.3	(131)
	Fraction IV precipitation	3.0		(129)
		4–5	4–5	(127)
			≥ 3.0	(131)
	Fraction V solution, first depth filtration		≥ 4.9	(131)
	Fraction V solution, second depth filtration		2.3	(131)
	Fraction V solution, membrane filtration		< 1	(131)
Immunoglobulin	Cryoprecipitation		1.0 ± 0.13	(128)
			< 1	(131)
	Fraction I		1.0 ± 0.08	(128)
Immunoglobulin	Fraction (I)+III precipitation	3.4		(129)
		4–5	4–5	(127)
			≥ 3.5 to ≥ 4.5	(128)
			≥ 3.7	(131)
	Fraction II solution, depth filtration		≥ 2.8	(131)
	IgG solution, first filtration	4.4		(129)
	IgG solution, second filtration	3.1		(129)
	IgG solution, filtrations combined	≥ 6.1		(129)
	IgG solution, nanofiltration	4.7		(129)

Ig, immunoglobulin.

Table 4. Reduction of Scrapie 263K by individual process steps used in the manufacture of coagulation factor concentrates.

Product	Process step	Scrapie reduction (Log_{10})		Reference
		Bioassay	Western blot	
Factor VIII concentrate	Cryoprecipitation	2.1		(111)
			< 1.0	(128)
			1.0	(131)
Factor VIII concentrate (immunopurified)	Immuno-affinity chromatography	4.4		(130)
	QAE-ion exchange chromatography	6.3		(130)
Factor VIII concentrate (ion exchange purified)	Precipitation + $Al(OH)_3$ adsorption		1.7	(131)
	DEAE-650M ion exchange		3.1	(131)
	Membrane filtration (0.22 µm)		1.0	(131)
Fibrinogen	Cryoprecipitation		1.0	(131)
	Precipitation + $Al(OH)_3$ adsorption		1.7	(131)
	DEAE-650M ion exchange		≥ 3.5	(131)
Factor IX concentrate	DEAE-ion exchange chromatography (a)		3.0	(131)
	DEAE-ion exchange chromatography (b)		3.0	(131)
	Heparin-sepharose chromatography		1.4	(131)
Thrombin	DEAE-ion exchange chromatography		3.0	(131)
	S-sepharose ion exchange chromatography		2.9	(131)

DEAE, diethylaminoethyl; QAE, quaternary aminoethyl.

Substantial removal of PrP^{RES} has been observed with certain depth filtres (131), suggesting that this type of operation may also contribute to the removal of prion agents. However, as these steps were studied individually, it did not yet become evident whether depth filtration would act in a manner complementary to precipitation.

Of the variety of *adsorption chromatography* procedures that are used in the manufacture of coagulation factor concentrates, most exhibit substantial removal of PrP^{Sc} or PrP^{RES} (Table 4), which is consistent with results from similar biopharmaceutical studies (126). Nevertheless, some PrP^{RES} remains detectable in the eluate fraction for most products (131). Whether this results from the high loading (overloading) of prion agent in these experiments or from the presence of a small 'fraction' of PrP^{RES}, which is desorbed more readily, remains to be determined.

Although these data indicate that process operations used in the manufacture of plasma products may well have a significant capability of removing prion agents, the accuracy and relevance of the experimental procedures used are still uncertain. Accurate results will be obtained only when the prion agent employed behaves in the same manner as human CJD agents and is present in the biophysical state in which it would occur naturally at each process step that is being examined. For example, material nearing the end of a purification process may be in a different state than that at the beginning, with the possibility that a soluble, nonaggregated 'fraction' of PrP^{Sc} may be the true challenge faced by end-stage steps such as nanofiltration. Other issues that remain to be clarified concern the relevance of scrapie as a model for vCJD; whether or not individual process steps may be complementary in their ability to remove the agent of vCJD, whether small-volume experimental procedures are accurate in simulating large-scale manufacturing operations, and whether the analytical methods used are sufficiently sensitive to identify the minimum quantity of prion agent that could remain infective in clinically relevant circumstances.

Dose response

The infectivity of prion agents is normally expressed in units of ID_{50}, with 1 ID_{50} being the quantity that causes disease in 50% of recipients. It has been estimated (137) that 1 ic ID_{50} equates to 10^5 molecules of PrP^{Sc}, which have been calculated to represents about 5×10^{-15} g of protein (138). Prion infectivity has been shown to exhibit a classical log-normal, sigmiodal dose–response curve in which all animals are infected when inoculated with high concentrations of prion agent but an increasing proportion fail to become infected as the dose is decreased, with eventually no infectivity being detected at very high dilutions. Examples of this behaviour include ic inoculation of mouse-adapted scrapie (139) and intraperitoneal injection of BSE-infected bovine brain (140) into mice. This dose–response profile is commonly observed in studies of viral infectivity and is generally believed to result when low quantities of infective material are distributed in the preparation from which the inoculum is taken; the probability of an infective dose being present in a sample is represented by a Poisson distribution (122, 141). Although the pathogenesis of prion diseases has still to be fully elucidated (142), it is believed that an element of chance may also determine whether or not an individual exposed to an infective dose will actually become infected (2), adding a further probabilistic element to the outcome.

Many recipients of blood products are treated more than once, with severe haemophiliacs and some recipients of immunoglobulin being treated repeatedly over many years. In estimating the risk to such individuals it is essential to know whether or not there is a threshold dose of PrP^{Sc} below which infection does not occur, or if very low doses of PrP^{Sc}, which individually would not cause infection, accumulate in a recipient until an infective dose is achieved. The former behaviour is generally regarded as being typical of infectious diseases, while the latter is more usually associated with exposure to toxic substances.

Some studies have examined the effect of multiple exposures by using oral administration of rodent-adapted scrapie. Diringer and co-workers (143) reported a higher incidence of infection in hamsters fed with 10 doses of scrapie extract rather than one dose, interpreting this as evidence for a cumulative effect. However, in each case, the degree of infectivity after 10 doses was consistent with what would be expected after 10 exposures to the infective risk represented by the individual dose. Kimberlin and Walker (144) found that mice given five doses of infective material exhibited an increased incidence of infection in proportion to the original risk multiplied five times; however, the titre of infectivity determined in brain tissue remained the same as that obtained with a single dose. By contrast, mice given a single, but five times greater dose, exhibited a higher titre of infectivity in brain tissue together with a shorter incubation period, which Kimberlin interpreted as evidence against the 'accumulated dose' hypothesis (113). Although these studies may be relevant to oral exposure to the BSE agent, the extent to which these findings will apply to other routes of administration is unclear, leaving some uncertainty over the nature of the dose response that would be associated with the use of blood products.

Conclusion

Despite considerable concern that established forms of CJD might be transmitted iatrogenically via blood or blood products, numerous studies have failed to establish any risk. Whether this results from the low prevalence of CJD worldwide, the low concentration of infectivity in plasma, dilution in the plasma pool, removal of infectivity during manufacturing processes, inefficient transmission in clinically relevant circumstances, a very long incubation period, or some combination of these factors, is not known (68, 122).

These observations cannot be extrapolated to the more recently discovered vCJD, as the prevalence of the disease is not known, and there is some evidence that the distribution of infective material in human tissue may differ from that of established forms of CJD (145). Given the long incubation period characteristic of TSE diseases (2), the actual risk (if any) of vCJD transmission by blood or blood products is unlikely to be known for many years.

Where an infective donation is used, the greatest risk of transmission of vCJD is likely to occur with individual blood components, such as platelets, plasma, and cryoprecipitate. Whether transmission of vCJD in these circumstances would be prevented by leukofiltration is not certain (68); therefore, further precautionary measures may be desirable, particularly in circumstances where the 'theoretical' risk is high.

Although an infective donation could be distributed more widely via large pool plasma products, there is growing evidence that the manufacturing processes employed in plasma fractionation may well be capable of substantially removing prion agents. Further work is required to confirm these observations and to determine the extent to which different process steps may or may not act in a complementary manner. Ideally, all processes should contain multiple complementary steps with a capacity for prion removal, with each step being robust, defined and controlled to the extent that prion removal can be assured. In addition, procedures should be developed that will ensure complete segregation between batches, so that more widespread contamination can be avoided should a vCJD-infected donation be processed.

According to growing consensus there is little or no risk of long-established forms of Creutzfeldt-Jakob disease (CJD) being transmitted to recipients of blood products. In contrast, the detection of abnormal prion protein in the tonsils of individuals with a variant of CJD infection has led to speculation that blood infectivity in this condition may be greater than in patients with CJD, and additional precautionary measures have been taken.

There is a pressing need for a sensitive and specific screening test suitable for testing blood donations, even if the prevalence of vCJD in the general population is found to be low; however, it may be some years yet before a suitable test is available (138). Assumptions concerning the effect of plasma fractionation on prion agents and the nature of the dose response have had a profound impact on the risk assessment undertaken on behalf of the UK government (53). These estimations illustrate the importance of these topics and the need for additional information before the actual risk of vCJD being transmitted by blood products can be estimated accurately.

Note added in proof:
In an experiment studying the transmissibility of BSE by blood transfusion, 19 scrapie-free sheep were transfused with blood taken from healthy sheep which had each previously been fed with 5 g of infected bovine brain. Although the study is still in progress, evidence of BSE infection has been reported in one sheep 610 days after being transfused with 400 mL of whole blood that had not been leukodepleted (146).

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