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研究報告の概要

スコットランド輸血サービスの高純度第Ⅷ因子濃縮製剤（Liberate）の製造工程における牛海綿状脳症（BSE）由来因子の除去能について測定した。マウス継代の301V株（BSE）の感染脳から調製されたミクロソーム分画を中純度の第Ⅷ因子工程液にスパイクした。この溶液をSD処理し、続いて陰イオン交換クロマトグラフィーで処理した。マウスバイオアッセイを用いて全分画の301V株の感染価を測定し除去能を出した。301V株の感染性はフィブリノーゲン分画において2.9 log、第Ⅷ因子分画において2.7 log除去された。添加した301V株の感染性の99%以上は、第Ⅷ因子溶出後のイオン交換カラムに残留したが、感染性の大部分は、2M-NaCl溶液でイオン交換樹脂を洗浄することで消失した。この結果は、vCJD感染性がフィブリノーゲンおよび第Ⅷ因子濃縮製剤の調製時に用いられるイオン交換プロセスにより十分に除去されることを示唆する。

報告企業の意見	今後の対応
高純度第Ⅷ因子濃縮製剤（Liberate）調製時の主要な精製段階における牛海綿状脳症（BSE）由来因子の挙動について測定した論文である。	これまでの疫学研究等では、ヒトにおいて、血漿分画製剤を介してvCJDが伝播するという証拠はない。また異常プリオンがアルブミン製剤の製造工程で効果的に除去されるとの報告もあるが、理論的リスクを完全には排除できないため、今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

クロスエイトM250
クロスエイトM500
クロスエイトM1000
血液を原料とすることに由来する感染症伝播等
理論的なvCJD等の伝播のリスク

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Vox Sanguinis (2004) 86, 92–99

ORIGINAL PAPER

Distribution of a bovine spongiform encephalopathy-derived agent over ion-exchange chromatography used in the preparation of concentrates of fibrinogen and factor VIII

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Vox Sanguinis

Background and Objectives The risk of haemophiliacs contracting variant Creutzfeldt-Jakob disease (vCJD) via treatment with factor VIII concentrates is not known. Therefore, in order to determine the extent to which the vCJD agent might be removed during the preparation of factor VIII concentrate, the partitioning of a bovine spongiform encephalopathy (BSE)-derived agent was measured over the main purification step used to prepare the Scottish National Blood Transfusion Service high-purity factor VIII concentrate (Liberate®).

Materials and Methods Murine-passaged BSE (strain 301V), in the form of a microsomal fraction prepared from infected brain, was used to 'spike' a solution of factor VIII of intermediate purity. The 'spiked' starting material was subjected to solvent-detergent treatment and then to anion-exchange chromatography with Toyopearl DEAE-650M. All fractions were tested for 301V infectivity using a murine bioassay, including the procedures used to clean the ion-exchange media after use.

Results BSE 301V infectivity was reduced by 2·9 $\log_{10}$ in the fibrinogen fraction and by 2·7 $\log_{10}$ in the factor VIII fraction. Over 99% of the added 301V infectivity remained bound to the ion-exchange column after elution of factor VIII. A large quantity of infectivity was subsequently removed by washing the ion-exchange media with 2 M NaCl. No further BSE 301V infectivity was detected in column eluates after treatment with 0·1 M NaOH or a second wash with 2 M NaCl.

Conclusions Results using a BSE-derived agent suggest that vCJD infectivity would be substantially removed by the ion-exchange process used in the preparation of fibrinogen and factor VIII concentrate. Although 301V infectivity remained bound to the ion-exchange matrix following elution of factor VIII, this appeared to be eliminated by the procedure used for cleaning the ion-exchange media after each use.

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Key words: Creutzfeldt–Jakob disease, chromatography, factor VIII, fibrinogen.

Introduction

Variant Creutzfeldt–Jakob disease (vCJD) is an incurable, fatal, neurodegenerative disorder of transmissible spongiform encephalopathy (TSE), of which there have been 150 confirmed or probable cases diagnosed since the condition was first reported [1]; 143 of these 150 subjects were resident in the UK at some time. Evidence that vCJD is caused by the TSE agent responsible for bovine spongiform encephalopathy (BSE) in cattle is convincing [2], with dietary exposure being the most probable route of transmission [3]. BSE originated in the UK [4] and has now been detected in cattle in 25 different countries, although 98% of all cases found

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have been in the UK [5]. Despite the relatively small number of confirmed or probable cases of vCJD, the absence of a suitable diagnostic screening test means that the number of people incubating the disease is not known, either in the UK or elsewhere.

Most TSEs in humans occur as sporadic Creutzfeldt–Jakob disease (sCJD), but 10–15% are linked to mutations of the PrP gene (e.g. familial CJD, Gerstmann Straussler Syndrome) and there have been several iatrogenic transmissions, most notably from contaminated preparations of human growth hormone. It was following the transmission of CJD via human growth hormone that blood products were identified as a possible route for the iatrogenic transmission of CJD [6], but no good evidence was found to support this hypothesis [7]. By contrast, there has been continuing concern that vCJD may be transmissible via blood or blood products [8,9], owing to the detection of abnormal prion protein (PrP^{Sc}) in the lymphoreticular tissue of patients with vCJD, but not in those with sCJD [10]. Experimental transmissions of BSE by transfusions of whole blood between sheep [11], and a probable human transmission of vCJD by a blood transfusion [12], have heightened these concerns. A number of precautionary measures have therefore been taken, including a ban on the manufacture of plasma derivatives from UK plasma [13] and, in many countries, deferral of blood or plasma donation from individuals who were resident in the UK during the BSE epidemic [14].

There is particular concern over the potential risk to patients treated repeatedly with plasma derivatives, as in the treatment of haemophilia A with factor VIII concentrates [15]. To establish the actual risk of vCJD being transmitted by this route it is necessary to determine the extent to which the agent responsible would be removed or inactivated by processes used to manufacture factor VIII concentrates. Previously, we examined the ability of a number of steps, used in the preparation of a high-purity factor VIII concentrate, to remove added PrP^{Sc}, finding the highest degree of PrP^{Sc} reduction over ion-exchange chromatography [16]. In this investigation, hamster-adapted scrapie was used as a source of prion agent and the partitioning of PrP^{Sc} was determined by immunochemical analysis. Although hamster-adapted scrapie is a well-established model for this type of study [17–23], the extent to which the findings can be extrapolated to the partitioning of vCJD infectivity remains uncertain. Therefore, we undertook a more substantial study of this ion-exchange purification step, using, as a high-titre TSE inoculum, a murine-passaged strain (301V) of BSE in the form of a microsomal fraction derived from infected brain tissue, as described previously [24]. In order to account fully for the fate of the added 301V, all output fractions from the ion-exchange process were examined for infectivity using a murine bioassay, including the procedures used to clean the ion-exchange media after each use.

Materials and methods

Preparation of a microsomal fraction of BSE 301V as the 'spiking' inoculum

In order to study the distribution of BSE-derived infectivity over ion-exchange chromatography, an aliquot of known infective material, in the form of microsomal inoculum, was added to a factor VIII solution of intermediate purity [16]. The procedure for the preparation of the microsomal inoculum was based on the method of Millson *et al.* [25]. Brain tissue (3 g), taken late in the clinical phase of disease from inbred VM mice infected with murine-passaged BSE (strain 301V), was suspended in 27 ml of phosphate-buffered saline (PBS), homogenized in a Dounce homogenizer and centrifuged at 700 *g* for 10 min at 4 °C. The pellet was resuspended in 10 ml of PBS and centrifuged again under the same conditions. The supernatants recovered from both procedures were pooled and centrifuged at 10 000 *g* for 7 min at 4 °C in order to sediment unbroken cells, large fragments of cells, mitochondria and cell nuclei. The translucent supernatant was removed carefully and centrifuged at 100 000 *g* for 1 h at 4 °C in order to sediment the microsomal fraction. The supernatant was discarded and the pellet was resuspended in 30 ml of PBS to provide the inoculum for 'spiking' the starting solution of intermediate purity factor VIII. The infectivity of this material was determined previously to be 7·25 $\log_{10}$ intracerebral infectious doses 50% (ID_{50})/ml [24].

Ion-exchange chromatography

The experimental procedure for determining the distribution of 301V infectivity over the ion-exchange chromatography process is shown schematically in Fig. 1.

Microsomal inoculum (10 ml) was added to a solution of factor VIII (102·7 ml), of intermediate purity, taken from a full-scale manufacturing batch that contained, 20 mM trisodium citrate, 2·5 mM calcium chloride, 109 mM NaCl and 4·5% (w/v) sucrose, as described previously [16]. Polysorbate-80 and tri(n-butyl) phosphate were then added to the solution to obtain concentrations of 1% v/v and 0·3% v/v, respectively, and the resultant mixture (108·6 ml) was stirred at 25 ± 1 °C for 18 h prior to processing by ion-exchange chromatography.

The ion-exchange procedure was also carried out as described previously [16]. Fourteen millilitres of Toyopearl DEAE-650M (TosohBiosep GmbH, Stuttgart Germany) was packed into a 10-mm diameter column (C10/20; Pharmacia, Upsala, Sweden) and equilibrated with 110 ml of buffer containing 120 mM glycine, 16 mM lysine, 10 mM trisodium citrate, 1 mM calcium chloride and 110 mM NaCl, pH 7·0.

Solvent/detergent-treated factor VIII solution (98·6 ml), containing 9·2% microsomal fraction by volume, was applied to the column at a flow rate of 78 ml/h, followed by 41 ml

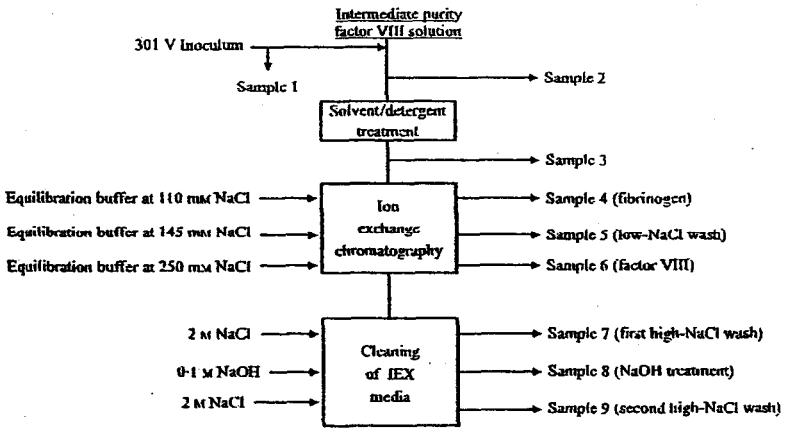

Fig. 1 Flow diagram of the processes over which partitioning of bovine spongiform encephalopathy (BSE) 301V infectivity was measured. IEX, ion-exchange chromatography.

of equilibration buffer, with the breakthrough (unadsorbed) fraction (139·8 ml) being collected (fibrinogen fraction). Forty-one millilitres of equilibration buffer, containing 145 mM NaCl, was then applied and the resultant wash fraction collected (low-NaCl wash). This was followed by 26 ml of equilibration buffer containing 250 mM NaCl, at a flow-rate of 48 ml/h, to elute factor VIII (factor VIII fraction).

Cleaning of the ion-exchange gel

Following collection of the factor VIII eluate, the chromatography bed was cleaned *in situ* by washing with 2 M NaCl, followed by 0·1 M NaOH and then again with 2 M NaCl. First, 25 ml of 2 M NaCl was applied to the column and the eluate (15·2 ml) was collected from the beginning of the 'salt front' (first high-NaCl wash). Subsequently, 0·1 M NaOH (70 ml) was applied to the column and an eluate (39 ml) was collected when the pH increased from 6·3 to > 12 (NaOH wash). When the application of 0·1 M NaOH was complete, the column was allowed to soak in NaOH for 1 h and then subjected to a second wash with 2 M NaCl (42 ml). An eluate volume of 8·1 ml was collected to capture the protein-containing fraction observed at this stage (second high-NaCl wash).

Determination of protein elution during the ion-exchange process

Throughout the ion-exchange procedure, the output from the column was monitored continuously by inline measurement of the solution optical density at a wavelength of 280 nm (OD_{280}) to detect total protein being eluted (Fig. 2).

Scale-down of the ion-exchange process

The small-scale ion-exchange procedure used in this study was designed to give yields and purification for factor VIII

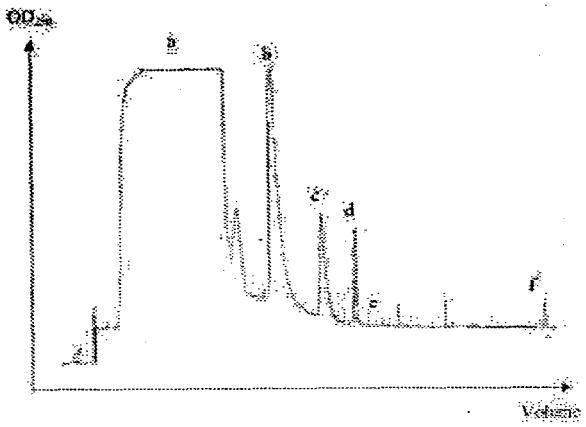

Fig. 2 Optical density of fractions eluted during ion-exchange chromatography of intermediate-purity factor VIII to which the bovine spongiform encephalopathy (BSE) 301V microsomal inoculum had been added. (a) Fibrinogen fraction (110 mM NaCl); (b) low-NaCl wash (145 mM NaCl); (c) factor VIII fraction (250 mM NaCl); (d) first high-NaCl wash (2 M NaCl); (e) NaOH wash (0·1 M NaOH); (f) second high-NaCl wash (2 M NaCl).

and fibrinogen equivalent to the full-scale process. Although the degree of scale-down was ≈ 1300-fold, all materials and surfaces were the same as in routine manufacture, except that chromatography eluates were collected into polypropylene containers rather than stainless steel vessels. The OD_{280} profile obtained in the presence of added 301V (Fig. 2) was the same as that obtained in the absence of 301V, both in the small-scale model and in the routine full-scale chromatography process, demonstrating the accuracy of down-scaling achieved.

Determination of BSE 301V infectivity

The BSE 301V infectivity of samples from the ion-exchange process was determined by bioassay. Samples for assay were diluted in saline and injected intracerebrally (20 µl) into

weanling VM mice. Animals were observed carefully for up to 547 days and scored for clinical signs, as described by Dickinson *et al.* [26]. BSE 301V infection in mice with clinical symptoms was confirmed by histopathological examination for TSE-specific brain vacuolation, as described by Bruce [27]. Surviving mice were killed between 539 and 547 days following injection and subjected to histopathological examination for evidence of asymptomatic infection.

Calculation of results

The degree to which mice were infected by a sample in the bioassay was used to calculate the titre of infectivity from the dose–response, using the Kärber method [28]. When there was no infectivity detected at the lowest dilution assayed, it was assumed that the maximum amount of infectivity present would be if the next lower dilution had 100% positive cases. In order to minimize the number of animals used, some samples were assayed at one dilution only. As the Kärber method is based on the dose–response, approximate titres of 301V were estimated from single-dilution data using the dose–response curve of the initial 'spiked' material, prior to the addition of solvent/detergent (sample 2). Infectivity titres were expressed as ID_{50}/ml. The extent to which infectivity was removed by chromatographic processing is expressed by a reduction factor (RF), calculated according to:

$$RF = \log_{10}[(T_0 \times V_0) \div (T_f \times V_f)]$$

Where T_0 = titre of 301V infectivity measured in the starting material, T_f = titre of 301V infectivity in the resultant fraction, V_0 = volume of starting material processed and V_f = volume of fraction recovered.

Results

The numbers of mice infected with 301V at different sample dilutions are given in Table 1, together with the time taken for the infection to be observed (mean incubation period). The original microsomal inoculum and the 'spiked' solution of intermediate-purity factor VIII, prior to and after treatment with solvent/detergent, all exhibited a high degree of infectivity. The mean incubation period for the microsomal fraction (sample 1), of 131 days at a 10^{-2} dilution (Table 1), was 11 days longer than we obtained previously [24]. However, the 301V titres measured in the solutions of intermediate-purity factor VIII, both before and after treatment with solvent/detergent (Table 2, samples 2 and 3), were

Table 1 Incidence of bovine spongiform encephalopathy (BSE) 301V infection in mice inoculated with different samples from an anion-exchange chromatography process used in the preparation of fibrinogen and factor VIII concentrate

Sample	Parameter	Sample dilution						
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
1. Microsomal inoculum	Mice infected/inoculated	12/12						
	incubation periods (days)[a]	131 ± 10						
2. FVIII solution, spiked	Mice infected/inoculated	6/6	6/6	5/6	4/6	0/12	0/10	0/11
	incubation period (days)	135 ± 11	145 ± 14	174 ± 12	198 ± 31	-	-	-
3. FVIII solution after S/D[b]	Mice infected/inoculated	6/6	6/6	4/5	5/6	0/6	0/6	0/6
	incubation period (days)	126 ± 8	147 ± 18	171 ± 2	186 ± 15	-	-	
4. Fibrinogen fraction	Mice infected/inoculated	7/12	0/11	0/11	0/12			
(110 mM NaCl)	incubation period (days)	204 ± 41	-	-	-			
5. Low-NaCl wash	Mice infected/inoculated	2/11						
(145 mM NaCl)	incubation period (days)	194 ± 5						
6. Factor VIII fraction	Mice infected/inoculated	12/12	6/11	1/12	0/11	0/12		
(250 mM NaCl)	incubation period (days)	166 ± 20	193 ± 9	245	-	-		
7. First high-NaCl wash	Mice infected/inoculated	6/6						
(2 M NaCl)	incubation period (days)	138 ± 9						
8. NaOH wash	Mice infected/inoculated	0/5						
(0·1 M NaOH)								
9. Second high-NaCl wash	Mice infected/inoculated	0/5						
(2 M NaCl)								

[a]Mean ± standard deviation.

[b]S/D, after treatment with solvent and detergent.

Table 2 Distribution of bovine spongiform encephalopathy (BSE) 301V infectivity across different fractions collected during the purification of fibrinogen and factor VIII by ion-exchange chromatography

Stage/fraction	BSE titre ID_{50}/ml (log_{10})	Volume of fraction (ml)	Total BSE infectivity in fraction, ID_{50} (log_{10})	% BSE infectivity in fraction	Reduction factor (log_{10})
1. Microsomal inoculum	7·3[a]	10·0	8·3		
Factor VIII process					
2. Factor VIII solution (spiked)	6·7	93·2	8·7		
3. Factor VIII solution after S/D[b]	6·8	98·6	8·8	100·00	
4. Fibrinogen fraction (120 mM NaCl)	< 3·8	139·8	5·9[c]	0·13	≥ 2·9
5. Low-NaCl wash (145 mM NaCl)	< 3·4[d]	41·0	5·0[c]	0·02	≥ 3·8
6. Factor VIII fraction (250 mM NaCl)	4·8	20·0	6·1	0·20	2·7
Column cleaning					
7. First high-NaCl wash (2 M NaCl)	6·4[d]	15·2	7·6	5·75	1·2
8. NaOH wash (0·1 M NaOH)	< 3·2	39·0	< 4·8	< 0·009	> 4·0
9. Second high-NaCl wash (2 M NaCl)	< 3·2	8·1	< 4·1	< 0·002	> 4·7

[a]Transmissible spongiform encephalopathy (TSE) titre obtained previously [24].
[b]S/D, after treatment with solvent and detergent.
[c]Maximum value on the assumption that 100% of animals would have been positive if the sample had been tested at a 10^{-1} dilution.
[d]Approximate TSE titre, estimated from bioassay at one dilution using the dose–response curve obtained with sample 2.
ID_{50}, infectious doses 50%.

consistent with the original level of infectivity, suggesting that aggregates may have formed during the frozen storage of the microsomal fraction and that full dispersion was only achieved after the microsomal fraction had been added to the solution of intermediate-purity factor VIII. Although there was a small apparent increase in 301V titre following solvent/detergent treatment (Table 2), this was well within the margin of error for TSE bioassay titrations. Nevertheless, a small increase in TSE titre is often detected after mild detergent treatment or other disaggregating treatment (e.g. sonication) and is probably a result of disaggregation, but may also occur as an effect of the efficiency of titration [29].

The three fractions recovered from the ion-exchange process, including the factor VIII fraction, all contained 301V infectivity. However, the quantity of infectivity present in each of these fractions was much less than that of the starting material. From these data it was calculated that with respect to the feedstock to ion-exchange chromatography, 301V infectivity was reduced by 2·9 log_{10} in the fibrinogen fraction and by 2·7 log_{10} in the factor VIII fraction (Table 2). It was also estimated that less than 0·4% of the 301V infectivity present in the feed to the ion-exchange process (sample 3) was recovered in the fractions collected up to and including the factor VIII fraction (Table 2), indicating that 99·6% of the added infectivity remained bound to the ion-exchange matrix following the recovery of factor VIII.

In the procedure used to clean the ion-exchange gel between uses, we found that a significant degree of infectivity desorbed into the first 2 M NaCl wash (Table 2). Subsequently, no 301V infectivity was detected in either the fraction recovered following treatment with 0·1 M NaOH nor in the second 2 M NaCl wash, despite further protein being desorbed at this point, according to the OD_{280} profile (Fig. 2, peak f).

Discussion

Despite considerable concern that haemophilia patients might be infected with vCJD by treatment with plasma products [15,30–33], few studies have been undertaken to determine how TSE agents distribute over processes used to manufacture factor VIII concentrates [7,34]. In modern factor VIII concentrates, a high degree of purification is obtained by chromatographic processing [35], yet information is available from only two studies concerning chromatographic purification of factor VIII, both of which employed scrapie 263K as the TSE model. Foster *et al.* [16] reported a 3·1 log_{10} reduction of PrP^{Sc} using a DEAE ion-exchanger, and Drohan has observed removal of a total of 3·5 log_{10} ID_{50} over QAE ion-exchange and 4·6 log_{10} ID_{50} over immunoaffinity chromatography [34]. Similar studies concerning ion-exchange purification of albumin and other plasma proteins have reported log_{10} reduction factors ranging from 2·2 to 5·2 [16,36,37].

If the agents responsible for BSE and vCJD share distinct properties, which are preserved on transmission to humans and mice, then 301V in mice may be a more suitable model than scrapie in experiments of this type. Our study with 301V was designed to allow a comparison to be made with scrapie 263K and we found that the reduction factors for fibrinogen

Table 3 Transmissible spongiform encephalopathy (TSE) reduction over ion-exchange chromatography using Toyopearl DEAE-650M: comparisons between scrapie 263K [16] and BSE 301V

Fraction from the ion-exchange process	TSE reduction factor (log_{10})[a]	
	Scrapie 263K[b]	BSE 301V[c]
Fibrinogen (non-adsorbed fraction)	≥ 3·5	2·9
Factor VIII fraction	3·1	2·7

[a]Reduction factors were calculated with reference to feedstock after treatment of the 'spiked' starting material with solvent/detergent.
[b]Feedstock 'spiked' with the microsomal fraction; distribution of abnormal prion protein (PRP^{Sc}) was determined by Western blotting.
[c]Feedstock 'spiked' with the microsomal fraction; distribution of infectivity was determined by murine bioassay.

and for factor VIII using 301V were similar to, but slightly lower than, those obtained using scrapie 263K (Table 3), probably because of the higher level of infectivity found in brain tissue in the 263K model [24]. In contrast to our work with 263K, some residual 301V was detected in the non-adsorbed (fibrinogen) fraction, probably as a result of the greater sensitivity of the bioassay compared with determination of PrP^{Sc} by Western blotting.

By examining all of the fractions resulting from the ion-exchange process, we were able to obtain a more comprehensive picture of where TSE infectivity was distributed than has previously been available for a chromatographic process. Nevertheless, it is not clear why some residual 301V infectivity partitioned into each of the three process fractions, the fibrinogen fraction, the low NaCl wash and the factor VIII fraction. Either the microsomal inoculum contained small subfractions of infectivity, each of which partitioned differently to the bulk of 301V, or some unbound infectivity remained in the flow-through (the fibrinogen fraction) and some bound infectivity desorbed into process fractions owing to the quantity of 301V exceeding the adsorptive capacity of the ion-exchange column. The latter explanation seems the most plausible, but further studies will be required to determine whether or not this was the case.

Treatment with solvent/detergent was included in order to model the overall manufacturing operation. Previously, the clearance of scrapie 263K was found to be similar over different ion-exchange processes, regardless of whether solvent/detergent treatment was included [16]. In the ion-exchange process studied here, both polysorbate-80 and tri(n-butyl) phosphate remain in the unadsorbed (fibrinogen) fraction and are therefore unlikely to have influenced 301V adsorption, other than perhaps by reducing the potential for PrP^{Sc} in the starting solution to aggregate.

In previous studies concerning the behaviour of TSE agents over chromatography, only a very small proportion of the scrapie was accounted for, leaving uncertain the fate of most of the TSE agent added. The high cost of chromatography reagents used in the manufacture of pharmaceutical proteins means that chromatographic media are reused many times, with batches of particularly expensive reagents being reused for a number of years. It has been shown that a virus bound to an ion-exchange matrix can remain adsorbed and then be eluted subsequently after multiple uses [38]. Therefore, it is important to determine if vCJD infectivity remains bound to a chromatographic matrix and, if so, whether or not this might result in the subsequent contamination of future batches of factor VIII concentrate processed using the same chromatography bed. Our results indicate that over 99% of the added 301V infectivity remained bound to the ion-exchange resin after factor VIII had been eluted. However, a significant amount of infectivity was removed in the first of two washes with 2 M NaCl, with no infectivity being detected subsequently, either after treatment with 0·1 M NaOH or after the second wash with 2 M NaCl. Given the degree of error associated with the murine bioassay, it is possible that all 301V infectivity remaining was desorbed by the first 2 M NaCl wash. However, Taylor has found that ≈ 6 logs of 301V infectivity was inactivated by 0·3 M NaOH in demineralized bone after the high load of lipid and protein associated with brain-tissue had been removed [39]. Therefore, it is conceivable that our treatment of the gel with 0·1 M NaOH, following cleaning with 2 M NaCl, may have inactivated some 301V infectivity that might otherwise have been detected in the protein fraction desorbed at the second 2 M NaCl wash. Whatever the precise mechanism responsible, the absence of detectable infectivity after a second wash with 2 M NaCl suggests that our cleaning procedure may have been effective in eliminating all of the 301V infectivity that remained bound after elution of factor VIII. If any 301V infectivity did remain bound to the gel following cleaning, the strength of adsorption involved suggests that this would be unlikely to elute into future preparations of factor VIII. Further studies are required to determine if any undetected infectivity remained bound to the ion-exchange matrix after completion of the cleaning procedures.

Our study examined the ion-exchange matrix on its first use. In our manufacturing operation this matrix is replaced after 10 uses, with no general change in performance being observed with this degree of use. However, further studies are required to determine if adsorption/desorption of TSE agents will differ over the lifetime of such reagents [40].

To interpret our results correctly, it is necessary to be aware of the limitations that concern studies of this type. There are two particular points to note: the nature of the TSE preparation used, and the sensitivity of the method of detection. In order to measure reduction factors over chromatographic purification of factor VIII, it is necessary to challenge the chromatography procedure with a relatively high titre of TSE

agent. We used a microsomal fraction for this purpose, for two reasons: first, by removing whole cells and large fragments, the method of preparation was similar to the separation of plasma from whole blood; and, second, to permit comparison of the results from this study with those from our earlier experiments with the scrapie agent in which a microsomal fraction was also used [16,24]. No specific measurements were performed to characterize the microsomal fraction, other than to titrate it for TSE infectivity. However, no significant TSE reduction has been observed over leucofiltration, using either endogenously infected murine plasma [41] or blood spiked with the microsomal fraction [42], indicating that, with respect to leucofiltration, the microsomal fraction contains PrP^{Sc} of a comparable state to that derived from an endogenous source. Nevertheless, the extent to which 301V infectivity from the micosomal fraction represents the vCJD agent as it would exist naturally at the intermediate stage of the factor VIII manufacturing process, has still to be established. Finally, our measurements on the procedure used to clean the ion-exchange matrix, and our inability to achieve an exact mass balance, were limited by the sensitivity of the murine bioassay (Table 2). This was constrained by dilution of the samples to make them suitable for intracerebral inoculation, the small volume of sample tested and the number of animals employed, which was minimized for ethical reasons.

Conclusions

This experiment has resulted in a number of important observations. First we have confirmed that ion-exchange chromatography can substantially remove a BSE-derived agent from preparations of fibrinogen and factor VIII concentrate. Second, most of the added TSE agent remained bound to the ion-exchange matrix after elution of factor VIII. Third, the cleaning procedure used to sanitize the ion-exchange matrix between uses was effective in eliminating a significant proportion, and possibly all, of the BSE-derived agent that remained bound after the elution of factor VIII. Finally, our results were similar to those obtained previously using hamster-adapted scrapie, suggesting that scrapie 263K may be a suitable TSE model for using to estimate the partitioning behaviour of the vCJD agent over ion-exchange chromatography.

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