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Removal of TSE agents by depth or membrane filtration from plasma products

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ABSTRACT

The removal of the abnormal form of prion protein i.e. PrP^{SC} by filtration steps in the plasma fractionation process has been investigated by immuno-Western blotting. Depth filtration has been shown to be capable of removing scrapie by 2–3 log from certain plasma product intermediates. These include cryoprecipitate supernatant, used for the manufacture of immunoglobulin and albumin, and albumin fraction V, by filtration using Pall Seitz or 3m Cuno depth filters respectively. However no significant removal occurred with immunoglobulin Fraction II after Cuno depth filtration. When 0.2 μm PVDF and Nylon membrane filters were tested, the removal of TSEs from 20% albumin was limited i.e. 0.6–1.3 log. However under protein free conditions using phosphate buffered saline, filtration was not effective in the case of a PVDF filter but very effective i.e. >2.9 log in the case of a Nylon filter.

1. Introduction

The agents that cause transmissible spongiform encephalopathies (TSEs) [1,2] in humans such as Creutzfeldt–Jakob disease (CJD) and the variant form of this disease (vCJD) may be transmitted by biological products. In the case of vCJD, a TSE that originally derived from bovine spongiform encephalopathy (BSE), there have been several possible cases of transmission by cellular blood products [3]. This raises the possibility that TSEs may be present in human plasma and thus potentially transmissible by plasma products such as factor VIII, immunoglobulin or albumin. Fortunately the properties associated with TSEs i.e. low solubility, a tendency to aggregate and to adhere to surfaces, suggests that steps used during the manufacture of plasma proteins such as precipitation, adsorption and chromatographic techniques should remove such agents [4]. Indeed this has been confirmed for many of the processes used in plasma product manufacture [5,6].

TSEs are highly resistant to both the physical or chemical methods of inactivation that are effective for other cellular microorganisms and viruses [7–9]. Furthermore, such methods cannot be applied to plasma products due to the susceptibility of the proteins to denaturation. Thus the elimination of TSE agents that may potentially be present will rely on process steps that physically remove these agents. Such steps may be standard manufacturing steps as well as steps deliberately incorporated into the process for TSE removal.

One approach for removing non-cellular microorganisms is to include filters of a very small-pore-size e.g. 20–50 nm [10–12]. Such filters have been shown to remove viruses from biological products and have been incorporated in many manufacturing processes. In addition, all manufacturing processes also include other types of filter during and at the end of the manufacturing process. These include depth or membrane filters used during the manufacturing process for removing particulates as well as 0.2 μm membrane filters used at the end of the process to sterilise the final formulated product. While these filters have a relatively large pore-size compared to those shown to have significant TSE removal capability, the properties of the TSE are such that significant removal on such filters may occur by mechanisms other than size.

In order to test whether such filtration steps using depth or membrane filters contribute to the removal of TSEs, small-scale filtration studies were carried out on selected steps. The steps selected were based on those used in the manufacture of immunoglobulin and albumin, two important products manufactured from human plasma. For these studies an immuno-Western blot method was used to determine the removal of the abnormal form of prion protein i.e. PrP^{SC}, the protein which has been shown to correlate with TSE infectivity [21].

2. Materials and methods

2.1. Scrapie spike

A microsomal preparation of the hamster adapted 263K strain of scrapie was used [13]. This form of spike was chosen as a compromise

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between a crude brain homogenate, that can prove difficult to filter, and extensively purified prion protein that can be present in an aggregated form. The scrapie spike was prepared by Douce homogenisation of infected brain tissue in phosphate buffered saline followed by centrifugation at 10,000 *g* for 7 min to remove cells and organelles. The microsomes remaining in the supernatant were then pelleted by centrifugation at 100,000 *g* for 1 h and resuspended in PBS. The material was extensively sonicated immediately before use to produce a homogenous preparation containing scrapie fragments of a relatively small size.

2.2. Filtration models

Intermediates and final product were provided by the Production Department at BPL and had been prepared by the Kistler and Nitschmann version of the ethanol fractionation process [24]. The small-scale filter test systems used were designed to imitate the full-scale manufacturing process as far as possible. In order to confirm the integrity of the filter during filtration, the pressure was monitored and confirmed to remain in the range defined for the process.

2.2.1. IgG: cryoprecipitate supernatant

Cryoprecipitate supernatant pH 7.7, and protein of 50 g/L used in the immunoglobulin process, was filtered through a Pall Seitz K250C filter (4–9μm) disc of 9.6 cm^2 surface area. This was carried out at ca 75% of the calculated down-scale process volume due to limitations in the volume of celite filter-aid that could be accommodated in the filter housing. Because of the large volume of cryoprecipitate supernatant involved, the amount of scrapie spike required was about 120 g. In order to reduce the amount required, the experiment was carried out in two stages. This has the advantage that the correct scale-down volume could be used. In the initial stage, the filter was conditioned with celite treated and filtered cryoprecipitate supernatant. The remaining cryoprecipitate supernatant was then spiked with 10% w/v scrapie and treated with 0.55%w/w celite by stirring at 2–4 °C for 90 min before being filtered at 2–4 °C and 1.5 bar through the Seitz filter.

2.2.2. Albumin: fraction V

Fraction V solution pH 7.4 and protein of 100 g/L from the albumin process was filtered through a small-scale 3M Cuno Zeta Plus filter (0.1μm) unit of 27 cm^2 surface area (BioCap BC0030A grade 90LP). Again filtration was carried out in two stages as described in 2.2.1. In the second stage, filtration with scrapie was conducted at 8–10 °C and 1.2–1.5 bar.

2.2.3. Immunoglobulin: fraction II

DENE treated fraction II intermediate pH 6.7 and protein of 65 g/L from the immunoglobulin process was filtered through a small-scale Cuno Zeta Plus filter (0.3μm) unit of 27 cm^2 surface area (BioCap BC0030A grade 60LP). The small-scale process was carried out at ca 65% of the calculated down-scale volume due to the different configurations of the manufacturing and small-scale systems. Filtration was carried out in two stages as described in 2.2.1. In the second stage, filtration with scrapie was conducted at 8–12 °C and 1–1.5 bar.

2.2.4. Membrane filtration

Studies on TSE removal by 0.2 μm sterilising grade membrane filters were also tested. For these studies 20% albumin pH 6.8–7.2 (Zenalb-20) was used. Albumin or PBS was filtered through a small-scale Millipore Durapore polyvinylidene fluoride (PVDF) or Pall Ultipore $Nylon_{66}$ filter of 9.6 cm^2 filter area. The conditions used were: load volume 42–49 ml, pressure 1.1–1.9 bar and temperature 20 °C. In addition to albumin, TSE removal from a protein free solution alone, i.e. PBS, was also tested.

2.3. PrP^{SC} assay

The titre of PrP^{SC} was determined by a Immuno-Western blot method that relies on the difference in susceptibility of the resistant infectious (PrP^{SC}) and normal (PrP^{C}) forms of the prion to protease digestion.

2.3.1. Sample preparation

All samples were ultracentrifuged at 108,000 *g* for 1 h and resuspended in PBS, either in the original volume or in a smaller volume to increase sensitivity, prior to assay. This process was used to reduce the level of background in the Western blotting assay process and thus to enable PrP^{SC} bands to be detected more easily. The samples were adjusted to a pH of 6.5–7.5, if necessary, and digested with Protease K (0.2–2.0 mg/ml) to remove any normal PrP^{C} that might be present. A 5-fold dilution series of each sample was then prepared.

2.3.2. Immuno-western blotting

The samples were denatured by boiling in 1% SDS with mercaptoethanol then run on a 15% polyacrylamide gel containing 0.1% SDS. After electrophoresis, the proteins were transferred to a nitrocellulose membrane by electroblotting using a semi-dry procedure. The membrane was washed with 25 mM Tris–HCl pH 7.6, 0.05% Tween 20 and 0.5 M NaCl and blocked with 5% skimmed milk powder in the same buffer. The PrP^{SC} protein bands were then detected using 3F4 monoclonal antibody [15], washed with 0.05% Tween 20 in PBS, followed by a rabbit anti-mouse secondary antibody labelled with horseradish peroxidase. Reactive bands were then detected using enhanced chemiluminescence. The end-point was taken as the last dilution where a PrP^{SC} band was just detectable in the test sample but was absent in the control unspiked sample.

A sample of high titre scrapie was also included and acted as a marker for the identification of the characteristic PrP^{SC} bands of about 27–30 kD. Prestained molecular weight markers of 14.3, 21.5, and 30 kD were also included. Negative controls comprising dilutions of unspiked product intermediate were also included and were used to confirm that there were no interfering bands present in the tests samples that comigrated with PrP^{SC}.

2.4. PrP^{SC} titre and reduction values

Relevant samples were run on the same gel in order to ensure the best comparison. The PrP^{SC} titre was expressed as a reciprocal of the dilution end-point and, after correction for the volume of the original sample, used to determine the total PrP^{SC} titre. The log reduction value was calculated by subtracting the total log PrP^{SC} remaining after filtration from that originally applied to the filter in the scrapie spiked product.

3. Results

3.1. Depth filtration

For these studies an immuno-Western blotting method was used to determine the titre of PrP^{SC} in test samples. In control studies, using a microsomal extract of scrapie infected brain, the principle PrP^{SC} bands of 27–30 kD could be detected and an end-point titre determined (Fig. 1A). Using this method the reduction in PrP^{SC} by filtration could be assessed. This is illustrated for the depth filtration of cryoprecipitate supernatant (Fig. 1B and C). Using the