The introduction of a novel prion protein (PrP^{Sc}) removal technology for the pharmaceutically licensed plasma Octaplas®

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Introduction

Four possible transmissions of variant Creutzfeldt-Jakob Disease (vCJD) through non-leukocyte depleted red blood cell concentrates in the UK [1-4] have made prion diseases a matter of concern in today's blood therapy. All manufacturers of plasma-derived biopharmaceuticals are requested to perform appropriate prion safety evaluations of their product portfolio, and an improved safeguarding in terms of risk for prion transmission is promoted when possible from a technological point of view and feasible in terms of appropriateness and quality outcome.

Octaplas® represents the first generation solvent/detergent (S/D) treated, human, coagulation-active plasma, and the product is manufactured without the use of any animal material and according to Good Manufacturing Practice (GMP) guidelines. The production process is straightforward and very reproducible. Cells and cell fragments are removed by a 1.0 µm depth-filtration. The S/D treatment is performed utilising 1.0% (w/w) tri-n-butyl-phosphate (TNBP) and 1.0% (w/w) Octoxynol at +30°C for 4 hours. TNBP is removed by liquid and Octoxynol by solid phase extraction. Finally, two filtration steps are performed (0.45 and 0.2 µm) in order to ensure sterility of the final product. It has previously been demonstrated that this manufacturing process is able to remove 2.5 log$_{10}$ cell-bound and free PrP^{Sc}, which already ensures a good safety margin for this plasma product in terms of prion transmission [5].

Specific affinity ligands have previously shown a major ability to remove PrP^{Sc} from blood components such as red blood cell concentrates [6,7]. The aim of our studies was to evaluate the effect of incorporating such a novel prion protein removal technology into the manufacturing process for Octaplas®, targeting a safety margin far beyond what has already been demonstrated for Octaplas®. A group of ligands with demonstrated strong affinity to prion protein was developed by the company PRDT (Pathogen Removal and Diagnostic Technologies Inc., US) and used here to assess the ligands' capacity to bind and thereby remove hamster-derived prion infectivity from Octaplas®. The best ligand candidate was immobilised on a resin matrix, which was used in a chromatography step as a single-use gel, i.e. no sanitisation and reuse. The technical implementation of the gel was done by Octapharma PPGmbH, Vienna, Austria. It was decided to incorporate the new PrP^{Sc} removal gel post-cell filtration and S/D treatment, at which the product matrix is clean from cells and debris that might contain or carry the pathogenic prions and prevent PrP^{Sc} from binding to the affinity ligands in the column.

Materials & Methods

A validated downscale model of the adapted manufacturing process was used as basis for all investigational studies. The 263K strain of hamster-adapted scrapie used in these experiments was supplied as a 10% crude brain homogenate (CBH) by the laboratory of Dr. Robert G. Rohwer (Baltimore, USA). A microsomal/cytosolic (MIC) fraction was prepared from this sample. The various pre-defined fractions were collected during laboratory processing and stored for subsequent detection and determination of PrP^{Sc} levels by standard Western blotting.

Results

Feasibility study for technology application

In order to investigate the feasibility of introducing this novel chromatography step into the Octaplas® manufacturing process, 0.5% sarkosyl treated spike material was prepared from CBH followed by centrifugation to remove debris. The supernatant (CBH$_{sark}$) was spiked into Octaplas® directly from the manufacturing process and applied to the prion removal resin in column format. Sarkosyl solubilised prion spike agents are widely used and yield a PrP^{Sc} preparation from which the membrane components have been removed, i.e. mimics well the nature of our target Octaplas® matrix. In these experiments, targeting a provoked breakthrough by PrP^{Sc} overloading, the binding capacity was confirmed to be dependent on the PrP^{Sc} load versus the amount of affinity ligands available in a reproducible manner (Table 1). Under a high PrP^{Sc} loading, a reduction factor of ≥ 3.0 log$_{10}$ could be demonstrated, utilising the relevant matrix from Octaplas® manufacturing. Within the accuracy of the assay, the total PrP^{Sc} loaded onto the column was quantitatively recovered – either in the experimentally applied 2 M NaCl wash or still bound to the gel.

Table 1. PrP^{Sc} removal during chromatography with a non-S/D treated spike

	Sample titre from end-point titration [log$_{10}$]	
Sample	5% CBH$_{sark}$ spike/ 9.5 ml gel	1% CBH$_{sark}$ spike/ 1.9 ml gel
Spiked start material	2.5	2.0
Flow-through 0-5 ml	≤ -0.5	≤ -0.5
Flow-through 5-10 ml	≤ -0.5	≤ -0.5
Flow-through 10-20 ml	0.5	1.0
Flow-through 20-50 ml	0.5	1.5
Flow-through 50-95 ml	0.5	1.5
2 M NaCl wash	2.0	1.5
Column gel	3.5	3.0

PrP^{Sc} removal at manufacturing conditions

For the total process studies, a MIC fraction was prepared from the CBH supernatant by centrifugation at 10,000 g. The MIC spike represents a worst-case PrP^{Sc} loading to the gel due to a high content of the smallest and most soluble form of PrP^{Sc}. It is assumed that the PrP^{Sc} binding sites on the gel will be occupied quicker by the MIC material than for a spike with the same overall concentration of PrP^{Sc}, but a larger particle distribution. In addition, a CBH from which the MIC fraction was removed (CBH$_{-mic}$) was prepared by centrifugation followed by re-suspension of the pellet in TBS. The CBH$_{-mic}$ spike was selected to investigate the binding of larger particle size PrP^{Sc}, i.e. those not contained in the MIC fraction.

Irrespective of the spike's nature, a complete PrP^{Sc} removal to below the limit of assay sensitivity was observed in the early flow-through from the column (Table 2). For the CBH$_{-mic}$ spike, a higher loss of spike material was observed for the steps prior to the column. However, this is consistent with the nature of this spike, which will contain larger membrane type aggregates. The pattern of provoked breakthrough also demonstrates slight differences between the two spike materials and is observed earlier with the MIC version. This may reflect an earlier saturation of available PrP^{Sc} binding sites by MIC due to the smaller prion aggregates present in this spike preparation, or it reflects the higher PrP^{Sc} loading onto the column due to the lower upstream loss of PrP^{Sc} compared to the CBH$_{-mic}$ case. Again the ≥ 3.0 log$_{10}$ reduction factor could be demonstrated and the amount of PrP^{Sc} recovered from the 2 M NaCl wash and gel show the substantial binding capacity of the affinity ligands. Based on the input of PrP^{Sc} and the sensitivity of the Western blot assay, it can be calculated that the PrP^{Sc} removal capacity per millilitre gel was at least 7.3 and 6.4 log$_{10}$ ID$_{50}$ for the MIC and CBH$_{-mic}$ spike, respectively.

Table 2. PrP^{Sc} removal during Octaplas® manufacturing with an S/D treated spike

	Sample titre from end-point titration [log$_{10}$]	
Sample	1% MIC spike	1% CBH$_{-mic}$ spike
Spiked Octaplas® after 1 µm filtration	2.5	2.0
After S/D treatment, liquid phase chromatography and depth-filtration	2.5	1.0
After solid phase chromatography	2.0	1.0
→ PRDT gel		
Flow-through 0-0.5 ml	≤ -0.5	≤ -0.5
Flow-through 0.5-5.0 ml	≤ -0.5	≤ -0.5
Flow-through 5.0-10 ml	≤ -0.5	≤ -0.5
Flow-through 10-20 ml	0.5	≤ -0.5
Flow-through 20-50 ml	1.5	0.5
2 M NaCl wash	3.0	2.0
Column gel	2.0	1.5

Competition by PrP^C

In theory, excessive amounts of PrP^C might be able to compete with PrP^{Sc} about the binding sites on the gel. Thus, a competition experiment was performed to address this particular issue. The normal concentration of PrP^C in plasma is estimated to be in the range of 10-100 ng/ml. The study therefore tested the ability of either normal Octaplas® or a solution of commercially available recombinant PrP^C at 2 µg/ml (i.e. at least 20 times higher than the concentration normally found in plasma) to elute gel-bound PrP^{Sc} from a pre-loaded column. It was concluded from these experiments that the PrP^C concentration expected to be found in the different Octaplas® batches have no impact on the ability of the column to bind and remove PrP^{Sc} from the product (data not shown).

Determination of PrP^{Sc} binding capacity per gel volume

In order to evaluate the PrP^{Sc} binding capacity per millilitre gel, studies were performed using sequential columns. In these experiments, the
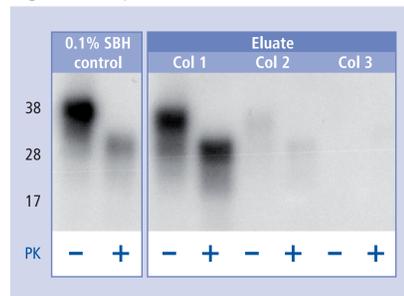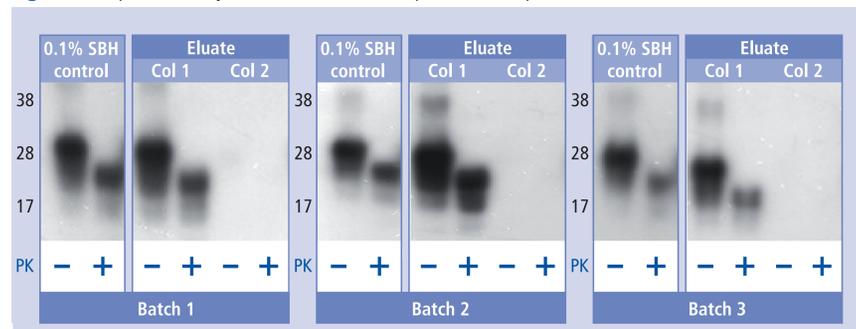
Figure 1. Sequential PrP^{Sc} removal

flow-through from the first column was applied onto the second column – and the second onto the third. The gel-bound PrP^{Sc} was quantified by densitometric reading of the Western blots and the binding capacity per column and millilitre gel was finally calculated versus the PrP^{Sc} input level. As a consequence, the gel's performance was in this case directly assessed as the amount of PrP^{Sc} bound to the gel and not by the amount of PrP^{Sc} remaining in the flow-through versus the input load in a traditional manner. In all cases the PrP^{Sc} binding capacity per millilitre gel was found to be 6.0 log$_{10}$ ID$_{50}$.

A complete binding of PrP^{Sc} by the first column could be confirmed by the absence of PrP^{Sc} in the second one. The results of the Western blotting from these high-load studies (Figure 1) indicated that the vast majority of the detectable signals were concentrated in column 1. The flow-through from column 1 contained occasionally some contamination of PrP^{Sc}, which was visualised as a very weak signal captured by the second gel, as shown in Figure 1. No signal was ever detected in column 3 indicating that all PrP^{Sc} had been removed way before this stage. Furthermore, the very solid PrP^{Sc} capture demonstrated was reproducible when different batches of gel were tested (Figure 2).

Prion safety margin for Octaplas® produced using affinity ligand column

The PrP^{Sc} binding capacity per millilitre gel was shown to be 6.0-7.3 log$_{10}$ ID$_{50}$. Thus, for the gel volume chosen (3.8 litre) for a standard Octaplas® batch size (380 litre), the total PrP^{Sc} capture is equivalent to at least 9.6 log$_{10}$ ID$_{50}$, which is equivalent to 9.4 log$_{10}$ ID (ID$_{50}$ x 0.69). In order to overload this removal capacity, every millilitre of such Octaplas® pools must contain more than 6,900 ID PrP^{Sc}. The prion causing vCJD has never been found in plasma of clinical cases of the disease. Up to 20 ID/ml plasma has, however, been found in relevant rodent models at the clinical stage of disease [8]. Thus, in theory one contaminated single plasmaphaeresis unit of 600 ml would cause a maximum PrP^{Sc} load of 0.03 ID/ml in the Octaplas® pool, i.e. ≥ 218,500 times (5.3 log$_{10}$) less than needed for overloading the prion removal gel capacity. Even with as many as 10 (1.6%) contaminated plasma units out of 630 plasmaphaeresis bags in an Octaplas® batch, the affinity ligand column is able to remove the total theoretical load of PrP^{Sc} with a safety margin higher than 21,850 fold (4.3 log$_{10}$).

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Figure 2. Reproducibility of PrP^{Sc} removal in sequential set-up

Conclusion

- The introduction of a specific prion protein removal column into the Octaplas® manufacturing process is technologically possible and will further improve the safety margin of Octaplas® in terms of prion diseases such as vCJD.

- Both small, soluble and larger, aggregated forms of PrP^{Sc} are rapidly and tightly bound with a high affinity to the disposable gel selected in a robust and reproducible manner.

- This technology, which is only applicable to controlled, industrial conditions, is able to capture more than 9.4 log$_{10}$ ID prion proteins from an Octaplas® batch.

- Even if 1.6% of all donors would carry PrP^{Sc}, the affinity ligand column is able to remove the total PrP^{Sc} load with a ≥ 21,850-fold (≥ 4.3 log$_{10}$) safety margin.

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