DISCUSSION:

A. Risk Assessment and Interpretation

Current FDA quantitative risk assessments use probabilistic models and Monte Carlo-based methods to sample individual values from statistical distributions of model inputs to produce thousands of theoretically possible individual scenarios that are combined into a single distribution describing the range of predicted outcomes for a risk (Vose 2000). The FDA December 2006 and June 2009 Risk Assessment Models are both intended to estimate the risk of vCJD infection for users of US-licensed pdFVIII as a function of product exposure for different assumed levels of infectious vCJD agent clearance during manufacturing of pdFVIII under each of two assumed levels of prevalence of vCJD infection in the UK (http://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/UCM095104.pdf; http://www.fda.gov/downloads/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/UCM095106.pdf).

First, after consultations with TSEAC, we outlined the successive steps involved in the manufacture of the product of concern and the events that would need to occur in each step for an infectious agent from a donor to reach the final product. The risk assessment utilizes a probability-based computer-based simulation model to evaluate successively the impact on vCJD risk of individual processes used to produce human pdFVIII beginning with plasma donation, vCJD infection prevalence in plasma donors, manufacturing steps, and, finally, differing levels of utilization of the product by various representative patient subpopulations. Input data for parameters used in the model, such as clearance of infectious vCJD agent by various steps in the manufacturing process and pdFVIII usage, are represented as statistical distributions that express the underlying uncertainties and variability. Each run of the model randomly samples one number from the distribution for each parameter; this is done thousands of times to generate a single distribution representing the final risk estimate that expresses, where possible, the accompanying uncertainty of these risk estimates. A sensitivity analysis, conducted by varying values of key parameters within the input range of the model and observing the effect on the predicted outcomes, determined that three major factors in the model greatly influenced potential vCJD risk: reduction of the infectious agent by the manufacturing process, intensity of pdFVIII utilization by the patient, and differing estimates of disease prevalence in the UK.

One of the most influential risk assessment parameters for vCJD is the manufacturing process, which may reduce the amount of vCJD agent in the final product or even or eliminate it. Because of the uncertainty and variability in the levels of vCJD clearance afforded during the manufacturing process for any pdFVIII product, the model evaluated two separate categories of reduction in infectivity that the product may have undergone during manufacturing including 4-6 $\log_{10}$, and 7-9 $\log_{10}$ reduction. These two categories are meant to span the possible range of uncertainty and variability in reduction of vCJD agent for US-licensed pdFVIII products. Based on currently available experimental studies, FDA believes that all US-licensed pdFVIII products probably achieve at least 4 $\log_{10}$-fold clearance of vCJD infectivity during manufacture.

Laboratory studies using model TSE agents have demonstrated reduction or elimination of TSE infectivity by certain types of manufacturing steps. Analogous to viral clearance studies, the capacity of a manufacturing process to clear TSE agents can be inferred from the results of experiments using validated scaled-down simulations of manufacturing processes and a well-characterized model TSE agent. FDA has recommended that such studies, if submitted for a labeling claim, supply the following information:

- Rationale for animal model selected to assay infectivity;
- Well-characterized bioassay for TSE infectivity;
- Rationale for selection of spiking preparation containing TSE agent;
- Characterization of spiking TSE agent;
- Demonstration of accurately scaled-down manufacturing processes (ordinarily evidenced by producing the desired active product);
- Reproducibility of experiments;
- Estimated $\log_{10}$ of TSE clearance by processing steps (log reduction factor [LRF]);
- Demonstration of "mass balance" (accounting for fate of all input infectivity);
- Demonstration that mechanistically similar clearance steps are or are not additive; and
- Account experimentally for "conditioning" of infectivity ("matrix" effect) because a prior step in the manufacturing process may affect the physical state of TSE agent and in turn affect downstream clearance.

In December 2006, the TSEAC discussed whether a minimum level of TSE clearance (total cumulative LRF) demonstrated by laboratory studies could be defined that enhances safety of plasma-derived products. The concept of a minimum level was agreeable to TSEAC. FDA proposed a total cumulative LRF of 6 log of clearance, based upon estimation of plasma infectivity derived from animal studies, results of the FDA 2006 Risk Assessment for pdFVIII, and including a margin of safety. However, TSEAC felt that, due to insufficient scientific certainty regarding the amounts of vCJD infectivity that might be present and the physical/chemical characteristics of infectivity in human plasma, it was not wise for FDA to recommend a firm minimum LRF (as demonstrated in experimental studies) that would guarantee the safety of pdFVIII prepared by any single manufacturing scheme. In addition, TSEAC members expressed concerns regarding the major limitations of studies involving spiked brain-derived TSE agents into blood or plasma for predicting clearance of endogenous vCJD agent from blood. There was agreement that while current exogenous spiking models have utility and enhance understanding of product safety, their limitations preclude recommending a specific minimum clearance level (http://www.fda.gov/ohrms/dockets/ac/06/transcripts/2006-4271t-unofficial.htm).

To date, FDA has allowed TSE clearance labeling claims for five plasma-derived products.[1] The minimum approved labeling claim has been for products manufactured by processes that demonstrated 6 $\log_{10}$ of clearance for model TSE agents in experimental studies. FDA has encouraged industry studies of pdFVIII manufacturing processes, which were presented to TSEAC in December 2006. The range of clearance offered by single production steps was 2.28 to 4.6 $\log_{10}$. Results of three of four studies were based on prion-protein-binding assays

[1] Carimune® NF, Panglobulin® NF, Privigen® Gamunex®, Thrombate III®

(detecting PrP^{TSE}) rather than infectivity assayed in known susceptible animals; a fourth study assessed clearance by infectivity bioassay (http://www.fda.gov/ohrms/dockets/ac/06/slides/2006-4271S1_00-index.htm). This raises questions as to the processes used for clearance of TSE infectivity in the manufacture of the "implicated" pdFVIII product received by the UK hemophilia patient with vCJD infection. Unfortunately, results of clearance studies are not available for that product.

Another major variable affecting potential risk is the quantity of product used by patients in different treatment groups. For purposes of this model, only patients with severe hemophilia A (HA) were considered because their higher use of product puts them at higher risk than patients with mild or moderate forms of the disease. Severe HA patients account for approximately 50% of the total HA population. Approximately 25% of all US HA patients use pdFVIII products, while most others use recombinant FVIII. (Data from a CDC-sponsored epidemiological study of HA patients were used to generate the statistical distribution of pdFVIII usage by patients [http://www.fda.gov/ohrms/dockets/ac/06/transcripts/2006-4271t1.pdf; http://www.fda.gov/ohrms/dockets/ac/06/transcripts/2006-4240t1.pdf; http://www.fda.gov/ohrms/dockets/ac/06/transcripts/2006-4240t2.pd]). Using these estimates, the risk assessment evaluated different treatment regimens. The five groups of patients requiring the largest amounts of product are, in increasing order of usage, (1) those treated with pdFVIII prophylaxis, (2) those treated with prophylaxis plus treatment for FVIII inhibitor, and (3) those treated with prophylaxis and having an inhibitor plus requiring induction of FVIII-immune tolerance. Patients generally requiring treatments with the smallest amounts of product are (4) those needing only episodic treatment, and (5) those needing episodic treatment plus having a FVIII inhibitor. We have also evaluated the potential risk to patients with severe von Willebrand disease (vWD), who are treated with pdFVIII containing von Willebrand Factor (vWF), because no recombinant vWF is available yet.

Results of the Updated Risk Assessment

Results from the updated FDA 2009 Risk Assessment Model for potential annual individual exposure and vCJD risk are shown in the Appendix in Table I. Results for potential annual individual exposure range from a low of approximately 1.7×10^{-7} iv ID_{50} per person per year (risk of 1 in 12 million) for patients who receive episodic treatment and have no inhibitor, to a higher potential exposure of approximately 1.6×10^{-4} iv ID_{50} per person per year (risk of 1 in 12,000) for patients on a prophylactic treatment regimen having both a FVIII inhibitor and induction of immune tolerance. A side-by-side comparison of the potential annual exposure estimates from FDA 2006 and 2009 Risk Assessments for all HA patients using a hypothetical pdFVIII product manufactured by a process that reduces the amount of infectious vCJD agent 4-6 $\log_{10}$-fold is shown in Appendix Table II. The comparison suggests that, even allowing for additional susceptibility of donors to vCJD, there is very little overall difference between the vCJD risk predicted by the FDA 2006 Risk Assessment Model and that generated by the updated FDA 2009 Risk Assessment Model. The biggest difference in the estimates (for 2009 versus 2006) was an approximately 4.5-fold difference (7.3×10^{-6} vs 1.57×10^{-6}) in annual exposure risk for patients who received a prophylactic treatment regimen and had both a FVIII inhibitor and needed treatment for immune

tolerance. However, even this difference is likely to have resulted from the large uncertainty and variability in the model inputs and probably does not represent a large increase in overall estimated vCJD risk.

A side-by-side comparison of model results from the FDA 2006 and 2009 Risk Assessments for the mean per patient risk at two levels of manufacturing process clearance of vCJD agent of 7-9 $\log_{10}$-fold and 4-6 $\log_{10}$-fold shows very little difference (Appendix Table III). As in Appendix Table II, the biggest difference in the estimates generated in 2009 versus 2006 was a less than 5-fold difference (1 in 270,000 vs 1 in 1.3 million) in annual exposure for patients who received a prophylactic treatment and additional treatment for both FVIII inhibitor and for induction of immune tolerance. Comparison of results from the FDA 2009 and 2006 Risk Assessments for vWD patients with severe disease (Appendix Table IV-A and IV-B) indicates little difference between estimates generated by each model. In some cases results in certain cells of Tables II, III, IV-A and IV-B indicate the risks for 2009 may appear lower or higher than the corresponding results for 2006. Because the results of each cell in each table are calculated independently of one another, and because of the significant uncertainty and variability in the model, one would expect this type of variation in the observed estimates of risk. Overall, even adding to a part of the FDA 2009 Risk Assessment the assumption that the entire UK population is susceptible to vCJD infection (the rest of the original FDA Risk Assessment in 2006 already assumed universal susceptibility), the results for 2009 and 2006 remain similar, supporting the same basic conclusions. Given the uncertainties of the models, it is still not possible to provide a precise estimate of the vCJD risk or to attempt to predict the actual risk to individual patients. As in 2006, the current results of the model continue to suggest that some users of pdFVIII might be exposed to the vCJD agent, so that there is a potential risk of infection, but that risk is likely to be extremely small, even for those patients using the largest amounts of product.

Interpretation

Results from the updated FDA 2009 vCJD pdFVIII Risk Assessment Model suggest that the risk of vCJD infection from US-licensed pdFVIII is likely to be extremely small but may not be zero. For US plasma donors, the major source of vCJD risk is dietary exposure during travel and/or residence in the UK, France, or other countries in Europe since 1980. Blood and plasma donor deferral criteria in place since 1999 have reduced the risk posed by donations from BSE-exposed and vCJD-exposed persons.

Manufacturing processes for human pdFVIII products are likely to reduce the quantity of vCJD agent, if present, but the level of reduction achieved by manufacturing steps is not precisely known. Clearance of TSE agents in manufacturing appears to vary among products, but clearance has not been measured in standardized studies that might allow more meaningful direct comparisons. Based on currently available experimental studies, it is estimated that pdFVIII products potentially undergo 4 $\log_{10}$ (10,000-fold) or greater reduction of the vCJD agent during the manufacturing process. Assuming a 4-6 $\log_{10}$ reduction in infectivity by the manufacturing process, modeling predicts that the potential risk per person per year for patients with severe HA using pdFVIII ranges from 1 in 12,000 for the higher vCJD infection prevalence estimate and high product usage, to as little as 1 in 12 million for the lower vCJD case prevalence estimate and low product usage. While higher levels of

clearance of vCJD infectivity by manufacturing are likely to reduce risk, it is not possible at this time to determine with certainty if a specific product may be more or less safe than another; that is due to the wide range of methods used for clearance studies, the results of clearance studies, and gaps in information. Although results of the model suggest that exposure to vCJD agent is possible, with a potential risk of infection that is likely to be extremely small, the model itself cannot provide a precise estimate either of the vCJD risk in general or of the actual risk to individual patients. Nonetheless, despite the uncertainties in the model, we believe this is information that patients and physicians might consider when making treatment decisions.

B. Risk Management Strategy

FDA's current risk management strategy for vCJD has evolved in response to emerging epidemiologic findings and basic scientific developments pertinent to the epidemic. The overall risk management strategy for vCJD includes the following:

- Deferral of donors at increased risk of vCJD based on epidemiological data, and withdrawal of certain products at increased vCJD risk:
  - Donor deferrals: Guidance since August 1999 (most recently updated in January 2002) to defer donors with "geographic risk," e.g., donors who visited or resided in countries where BSE prevalence is higher; deferral of donors who used UK-sourced bovine insulin; deferral of donors transfused in the UK since 1980 (note also that a draft guidance published in August 2006 proposed deferral of donors transfused in France since 1980); and
  - Withdrawal of vCJD-implicated blood components and plasma derivatives is recommended if a donor is diagnosed with vCJD (which has not occurred).

- Facilitating development, validation, and information sharing (including product labeling) regarding the performance of manufacturing processes in clearance of TSE agents from blood products:
  - FDA reviews requests for TSE clearance labeling claims which may be approved if detailed, validated TSE clearance study data are provided.
  - On September 18, 2006, FDA discussed with TSEAC the feasibility and scientific value of standardized assessments of TSE clearance in the manufacturing processes for pdFVIII. The topic will be addressed again at this meeting.

- Facilitating development of candidate donor screening and diagnostic tests for vCJD and other TSEs:
  - FDA has held meetings with candidate test kit manufacturers to discuss developmental pathways.
  - A public discussion of validation for donor screening tests for vCJD and other TSEs was held with the TSEAC on September 19, 2006.

- Risk assessment and communication to inform patients and physicians about the current scientific understanding regarding vCJD risk from blood products and to help inform treatment decisions:

- FDA has engaged in periodic reassessment of TSE epidemiology and pathogenesis to determine whether guidance/policies need to be revisited in light of new information.
- FDA performed risk assessments for potential exposure to vCJD in investigational pdFXI made from plasma donated in the UK, and for US-licensed pdFVIII made from plasma donated in the US.
- FDA developed and posted risk communication materials on the FDA website.
- FDA communicates with patients organizations when new events occur regarding vCJD.
- FDA encourages physicians and patients to consider this risk in making treatment decisions.

Questions for the Committee:

Based on an updated risk analysis, FDA continues to believe that the risk of variant Creutzfeldt-Jakob disease (vCJD) to patients who receive US-licensed plasma-derived coagulation factor VIII (pdFVIII) products is likely to be extremely small, although we do not know the risk with certainty.

1. Should the recent report from the UK Health Protection Agency, attributing a case of vCJD infection to treatment 11 years earlier with a "vCJD-implicated" pdFVIII, alter FDA's interpretation of the risk for US-licensed preparations of pdFVIII?

2. If so, should FDA consider:
   a. Recommending additional risk-reducing steps for manufacture of plasma derivatives (e.g., modifications to current donor deferral policies)?
   b. Recommending revised warning labels for plasma derivatives?
   c. Recommending modifications to FDA's public communications (e.g., to Web postings) regarding the risk of vCJD associated with the use of FDA-licensed plasma derivatives?

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Appendix with Tables I through IVB

Table I. Updated FDA 2009 Model results for all hemophilia A patients with severe disease using hypothetical pdFVIII produced by process with 4-6 Log_{10} Reduction Factor (LRF) of vCJD infectivity: Potential mean per person exposure to vCJD iv ID_{50} and mean per person vCJD risk per year

				4 - 6 Log_{10} Reduction Factor (LRF)			
				Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)		*Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton et al (2004)*	
Treatment Regimen	Inhibitor Status	Est. Total Number patients in US	Mean quantity FVIII used per person per year (5^{th} - 95^{th} perc)	Mean exposure to vCJD iv ID_{50}* per person per year (5^{th} - 95^{th} perc)	Mean** potential vCJD risk per person per year (5^{th} - 95^{th} perc)	Mean exposure to vCJD iv ID_{50}* per person per year (5^{th} - 95^{th} perc)	Mean** potential vCJD risk per person per year (5^{th} - 95^{th} perc)
Prophylaxis	No Inhibitor	578	157,949 IU (21242, 382316)	4.9×10^{-7} (0-0)	1 in 4.0 million (0-0)	4.5×10^{-5} (0 - 2.1×10^{-4})	1 in 44,000 (0 - 1 in 4,700)
	With Inhibitor – No Immune Tolerance	63	190,523 IU (26956, 447639)	7.5×10^{-7} (0-0)	1 in 2.7 million (0-0)	5.4×10^{-5} (0 - 2.6×10^{-4})	1 in 37,000 (0 - 1 in 3,900)
	With Inhibitor – With Immune Tolerance	62	558,700 IU (33235, 1592943)	7.3×10^{-6} (0-0)	1 in 270,000 (0-0)	1.6×10^{-4} (0 - 7.4×10^{-4})	1 in 12,000 (0 - 1 in 2,700)
Episodic	No Inhibitor	946	85,270 IU (4633, 244656)	1.7×10^{-7} (0-0)	1 in 12 million (0-0)	2.5×10^{-5} (0 - 1.1×10^{-4})	1 in 81,000 (0 - 1 in 18,000)
	With Inhibitor	151	160,458 IU (5314, 488906)	8.6×10^{-7} (0-0)	1 in 2.3 million (0-0)	4.6×10^{-5} (0 - 2.0×10^{-4})	1 in 43,000 (0 - 1 in 9,800)

*iv ID_{50} represents the probability that 50% of those exposed to 1 ID_{50} intravenously may become infected with vCJD.

**Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Total mean quantity iv ID_{50} per year x 0.5 (50 % chance infection from ID_{50})

Table II. Comparison of FDA 2006 and 2009 Risk Assessment results estimating mean potential annual exposures to vCJD iv ID_{50} for all hemophilia A patients using hypothetical pdFVIII produced by process with 4-6 LRF of vCJD infectivity

					4 - 6 Log_{10} Reduction Factor (LRF)	
					Model Output for LOWER vCJD Case Prevalences based on Clark and Ghani (2005)	*Model Output for HIGHER vCJD Infection Prevalences based on Hilton et al (2004)*
Treatment Regimen	Inhibitor Status	Total Number patients in US	Mean quantity FVIII used per person per year (from FDA 2006)	Year FDA Risk Assessment Conducted	Mean exposure to vCJD iv ID_{50}* per person per year	Mean exposure to vCJD iv ID_{50}* per person per year
Prophylaxis	No Inhibitor	578	157,949 IU	2009 / 2006	4.9×10^{-7} / 4.99×10^{-7}	4.5×10^{-5} / 3.67×10^{-5}
	With Inhibitor – No Immune Tolerance	63	190,523 IU	2009 / 2006	7.5×10^{-7} / 4.21×10^{-7}	5.4×10^{-5} / 4.86×10^{-5}
	With Inhibitor – With Immune Tolerance	62	558,700 IU	2009 / 2006	7.3×10^{-6} / 1.57×10^{-6}	1.6×10^{-4} / 1.30×10^{-4}
Episodic	No Inhibitor	946	85,270 IU	2009 / 2006	1.7×10^{-7} / 2.12×10^{-7}	2.5×10^{-5} / 1.91×10^{-5}
	With Inhibitor	151	160,458 IU	2009 / 2006	8.6×10^{-7} / 2.49×10^{-7}	4.6×10^{-5} / 4.19×10^{-5}

*iv ID_{50} represents the probability that 50% of those exposed to 1 ID_{50} intravenously may become infected with vCJD.
**Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Total mean quantity iv ID_{50} per year x 0.5 (50 % chance infection from ID_{50}.

TABLE III. Comparison of results from FDA 2006 and 2009 Risk Assessments for mean potential per-patient vCJD risk for all hemophilia A patients using hypothetical pdFVIII at two levels of manufacturing process reduction in vCJD agent infectivity (7-9 LRF and 4-6 LRF) and assuming both LOWER and HIGHER prevalence estimates

					7 - 9 Log_{10} Reduction Factor (LRF)		4 - 6 Log_{10} Reduction Factor (LFR)	
					Model Output for LOWER vCJD Case Prevalences based on Clark and Ghani (2005)	*Model Output for HIGHER vCJD Infection Prevalences based on Hilton et al (2004)*	*Model Output for LOWER vCJD Case Prevalences based on Clark and Ghani (2005*	*Model Output f HIGHER vCJ Infection Prevalences based on Hilto et al (2004)*
Treatment Regimen	Inhibitor Status	Total Number patients in US	Mean quantity FVIII used per person per year (from FDA 2006)	Year FDA Risk Assessment Conducted	Mean potential vCJD risk per person per year	Mean potential vCJD risk per person per year	Mean potential vCJD risk per person per year	Mean potentia vCJD risk per person per year
Prophylaxis	No Inhibitor	578	157,949 IU	2009	1 in 5.4 billion	1 in 44 million	1 in 4.0 million	1 in 44,000
				2006	1 in 4.1 billion	1 in 50 million	1 in 4.0 million	1 in 54,000
	With Inhibitor – No Immune Tolerance	63	190,523 IU	2009	1 in 2.8 billion	1 in 37 million	1 in 2.7 million	1 in 37,000
				2006	1 in 3.5 billion	1 in 40 million	1 in 4.8 million	1 in 41,000
	With Inhibitor – With Immune Tolerance	62	558,700 IU	2009	1 in 200 million	1 in 12 million	1 in 270,000	1 in 12,000
				2006	1 in 551 million	1 in 15 million	1 in 1.3 million	1 in 15,000
Episodic	No Inhibitor	946	85,270 IU	2009	1 in 12 billion	1 in 81 million	1 in 12 million	1 in 81,000
				2006	1 in 3.2 billion	1 in 100 million	1 in 9.4million	1 in 105,000
	With Inhibitor	151	160,458 IU	2009	1 in 1.8 billion	1 in 44 million	1 in 2.3 million	1 in 43,000
				2006	1 in 4 billion	1 in 50 million	1 in 8million	1 in 23,000

Table IV-A. Comparison of results from FDA 2006 and 2009 Risk Assessments for vonWillebrand disease (vWD) patients with severe disease: Predicted potential annual exposures to vCJD agent in iv ID_{50} and vCJD risk assuming 4-6 LRF by manufacturing process

YOUNG vWD (≤ 15 yrs of age)

				4- 6 Log_{10} Reduction Factor (LRF)			
				Model Output for LOWER vCJD Case Prevalences based on Clark and Ghani (2005)		*Model Output for HIGHER vCJD Infection Prevalences based on Hilton et al (2004)*	
	Est. Total Number patients in US	Mean quantity product used per person per year (from FDA 2006)	Year FDA Risk Assessment Conducted	Mean exposure to vCJD iv ID_{50}* per person per year (5^{th} - 95^{th} perc)	Mean** potential vCJD risk per person per year (5^{th} - 95^{th} perc)	Mean exposure to vCJD iv ID_{50}* per person per year (5^{th} - 95^{th} perc)	Mean** potential vCJD risk per person per year (5^{th} - 95^{th} pe...
Prophylaxis	39	165,713 IU	2009 / 2006	3.6×10^{-7} / 4.3×10^{-7}	1 in 5.6 million / 1 in 4.7 million	3.4×10^{-5} / 3.81×10^{-5}	1 in 59,000 / 1 in 52,000
Episodic	60	11,045 IU	2009 / 2006	2.7×10^{-8} / 4.14×10^{-8}	1 in 75 million / 1 in 48 million	3.2×10^{-6} / 2.06×10^{-6}	1 in 630,000 / 1 in 971,000

Table IV-B. Comparison of results from FDA 2006 and 2009 Risk Assessments for vonWillebrand disease (vWD) patients with severe disease: Predicted potential annual exposures to vCJD agent in iv ID50 and vCJD risk assuming 4-6 LRF by manufacturing process

ADULT vWD (> 15 yrs of age).

				4- 6 Log_{10} Reduction Factor (LRF)			
				Model Output for LOWER vCJD Case Prevalences based on Clark and Ghani (2005)		*Model Output for HIGHER vCJD Infection Prevalences based on Hilton et al (2004)*	
Prophylaxis	73	186,880 IU	2009 / 2006	5.2×10^{-7} / 4.89×10^{-7}	1 in 3.9 million / 1 in 4.1 million	4.1×10^{-5} / 4.32×10^{-5}	1 in 49,000 / 1 in 46,300
Episodic	78	86,923 IU	2009 / 2006	2.2×10^{-7} / 1.99×10^{-7}	1 in 9.3 million / 1 in 10million	2.22×10^{-5} / 1.90×10^{-5}	1 in 75,000 / 1 in 53,000#

#The original risk estimate for this cell in the FDA Risk Assessment of 2006 (FDA 2006) was incorrect – the corrected estimate is provided in this table

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識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2009 年 7 月 3 日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称			研究報告の公表状況	Prion removal effect of a specific affinity ligand introduced into the manufacturing process of the pharmaceutical quality solvent / detergent (S/D)-treated plasma OctaplasLG. A. Neisser-Svaeet et al, Vox Sanguinis 97, 226-233. 93, (2009).	公表国 オーストリア	
販売名（企業名）						

研究報告の概要

異常プリオン蛋白（PrP^{Sc}）と特異的に結合する親和性リガンドを用いた新しいアフィニティクロマトグラフィの技術が開発された。本試験では，変異型クロイツフェルト・ヤコブ病（vCJD）伝播リスクに対する安全性を向上させる目的で，solvent/detergent 処理血漿分画製剤オクタプラスの製造過程に本法を導入し，各処理段階における PrP^{Sc} 除去効果を検証した。ハムスター順化スクレイピー263K 株感染ハムスターより得られた 10%未精製脳ホモジネート（CBH）を遠心分離し，ミクロソーム／サイトゾル画分（MIC）と $CBH_{(-MIC)}$ を精製した。オクタプラスを MIC あるいは $CBH_{(-MIC)}$ にスパイクさせ，親和性リガンド固定樹脂カラムに添加した。分画サンプル中の PrP^{Sc} をウエスタンブロットにて同定・定量した結果，MIC スパイクサンプルでの reduction factor は≧3.0 log_{10} 相当であった。MIC および $CBH_{(-MIC)}$ スパイクサンプルのリガンドゲル 1mL 当たりの PrP^{Sc} 除去能は非常に高く，7.3 および 6.4log_{10} ID50/mL であった。0.01%に希釈した CBH_{Sark} のスパイクサンプルを 3 個の連続した樹脂カラムに添加し，ウエスタンブロットを行ったところ，3 個目の樹脂カラムからは PrP^{Sc} は全く検出されなかった。PrP^{Sc} 除去能は 6.0log_{10} ID50/mL リガンドゲルであり，これは製造工程においては約 10log_{10} ID50/カラムに相当する除去率であった。ハムスタースクレイピー263K 株，ヒト vCJD 株，ヒト散発性 CJD 株，Gerstmann-Straussler Scheinker 病マウス Fukuoka 株由来 PrP^{Sc} に対するリガンド特異性を検討した結果，全ての株由来 PrP^{Sc} がウエスタンブロットによって検出され，ヒト由来 PrP^{Sc} に対するリガンド特異性が確認された。本研究では，親和性リガンドを用いた方法により非常に高い PrP^{Sc} 除去効果が得られることが示された。また，本法をオクタプラスの製造過程に導入することは技術的に可能であり，vCJD 感染の観点からも本法導入によるオクタプラスの安全域の拡大が期待される。

使用上の注意記載状況・その他参考事項等

BYL-2009-0387

報告企業の意見	今後の対応
本論文では，効率的に異常プリオン蛋白（PrP^{Sc}）除去可能なアフィニティクロマトグラフィが開発されたことが発表されたが，この工程は血漿分画製剤の製造過程に導入することが可能であるという点で，製造工程における異常プリオン除去能力をさらに向上させることができる可能性が高く，導入が非常に期待される開発である。	現時点で新たな安全対策上の措置を講じる必要はないと考える。 本稿で報告されたような，導入が可能と思われるウイルス除去法に関して今後とも情報収集に努める。

Vox Sanguinis (2009)

ORIGINAL PAPER

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Prion removal effect of a specific affinity ligand introduced into the manufacturing process of the pharmaceutical quality solvent/detergent (S/D)-treated plasma OctaplasLG®

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Vox Sanguinis

Background and Objectives A new chromatographic step for the selective binding of abnormal prion protein (PrP^{Sc}) was developed, and optimization for PrP^{Sc} capture was achieved by binding to an affinity ligand attached to synthetic resin particles. This step was implemented into the manufacturing process of the solvent/detergent (S/D)-treated biopharmaceutical quality plasma Octaplas® to further improve the safety margin in terms of risk for variant Creutzfeldt–Jakob disease (vCJD) transmission.

Materials and Methods Intermediates and Octaplas® final container material, spiked with hamster brain-derived PrP^{Sc}-containing fractions, were used for experiments to establish the feasibility of introducing this novel chromatography step. The binding capacity per millilitre of ligand gel was determined under the selected manufacturing conditions. In addition, the specificity of the ligand gel to bind PrP^{Sc} from human sources was investigated. A validated Western blot test was used for the identification and quantification of PrP^{Sc}.

Results A reduction factor of ≥ 3·0 $\log_{10}$ could be demonstrated by Western blotting, utilizing the relevant Octaplas® matrix from manufacturing. In this particular cell-free plasma solution, the PrP^{Sc} binding capacity of the selected gel was very high (≥ 6 $\log_{10}$ ID_{50}/ml, equivalent to roughly 10 $\log_{10}$ ID_{50}/column at manufacturing scale). The gel binds specifically PrP^{Sc} from both animal (hamster and mouse) and human (sporadic and variant CJD) sources.

Conclusion This new single-use, disposable PrP^{Sc}-harvesting gel ensures a very high capacity in terms of removing the pathogenic agent causing vCJD from the new generation OctaplasLG®, in the event that prions can be found in plasma from donors incubating the disease and thereby contaminating the raw material plasma used for manufacturing.

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Key words: affinity ligand chromatography, OctaplasLG®, prion safety, PrP^{Sc}, vCJD.

Introduction

In the last few years, four probable transmissions of variant Creutzfeldt–Jakob disease (vCJD) through non-leucocyte depleted red blood cell concentrates in the UK [1–4], as well as the first probable case of vCJD through a plasma-derived factor concentrate [5], have made prion diseases a matter of concern in today's blood therapy.

A number of actions have been implemented by regulatory authorities, such as requiring that all manufacturers of plasma-derived biopharmaceuticals should perform appropriate prion safety evaluations of their product portfolio. Different

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manufacturing steps have been demonstrated to provide significant removal, either of prion infectivity or the disease-associated marker PrP^{Sc} [6]. Specific affinity ligands designed to bind prions have previously shown a significant capacity to remove PrP^{Sc} and associated infectivity from blood components such as red blood cell concentrates [7–9]. Such specific affinity ligands have until now not been investigated for the removal of PrP^{Sc} in plasma-derived biopharmaceuticals such as Octaplas®.

Octaplas® is the first generation solvent/detergent (S/D)-treated, human, coagulation-active plasma. The production process is straightforward and very reproducible. Cells and cell fragments are removed by a 1·0 μm filtration step at the front-end of the process. The S/D treatment is performed utilizing 1·0% (w/w) tri-n-butyl-phosphate (TNBP) and 1·0% (w/w) Octoxynol-9. TNBP is subsequently removed by oil and Octoxynol-9 by solid phase extraction. Finally, two filtration steps are performed (0·45 and 0·2 μm) to ensure sterility of the final product.

It has already been demonstrated that the current Octaplas® manufacturing process is able to remove 2·5 $\log_{10}$ cell-bound and free PrP^{Sc}, when using a chronically infected cell line as spike material, which in itself ensures a good safety margin for this plasma product in terms of prion transmission [10]. The implementation of an additional orthogonal prion removal step would further enhance the safety of Octaplas® in this respect. The company Pathogen Removal and Diagnostic Technologies Inc. (PRDT, NY, USA) has developed a group of ligands, coupled to a standard resin base, which have demonstrated strong affinity for the prion.

The studies reported in this paper were designed to determine the potential for prion removal by a specific affinity ligand implemented into the new generation OctaplasLG® (LG, ligand gel) manufacturing process. To prevent potential interference of the non-homogeneous plasma product (e.g. possibly containing cells and cell debris) with the binding of PrP^{Sc} to the affinity ligand, it was decided to incorporate the new prion removal resin post-cell filtration and S/D treatment, at which point the product is clean from cells and debris that might contain or carry the pathogenic prions. The technical implementation of the ligand resin was performed by Octapharma PPGmbH, Vienna, Austria.

Materials and methods

Spike material preparations

The 263K strain of hamster-adapted scrapie used in the experiments was supplied as a 10% crude brain homogenate (CBH) by the laboratory of Dr Robert G. Rohwer (Baltimore, MD, USA). A microsomal/cytosolic (MIC) fraction was prepared from the 10% CBH following the preparation procedure established for various TSE sub-cellular fractions (the CBH was centrifuged at 10 000 *g* for 8 min at ambient temperature and the supernatant was separated from the pellet and harvested as the MIC fraction) [11]. For studies on the robustness of PrP^{Sc} removal, the pellet from the above centrifugation was used as the spike [$CBH_{[-MIC]}$] after re-suspended at a ~10% concentration in tris-buffered saline (TBS) or phosphate-buffered saline (PBS). The $CBH_{[-MIC]}$ fraction contained the large membrane fragments and tissue not present in the MIC fraction, which was mostly consistent of more soluble and presumably smaller PrP^{Sc} components.

The studies shown in Figs 2–4, as well as the supporting feasibility studies, were performed with a Sarkosyl-treated spike material. CBH was treated with 0·5% Sarkosyl for 30 min on ice. The solution was centrifuged at 13 000 *g* for 10 min at room temperature to remove debris. The supernatant (CBH_{Sark}) was used as the spike [8].

Determination of PrP^{Sc}

The proteinase K (PK) digestion and Western blot assay used for the detection of PrP^{Sc} were either performed as described by Gregori L *et al.* [8] or with some minor modifications – where Triton X-100 instead of sodium dodecyl sulphate (SDS) was used as detergent during the PK digestion step, and where the polyacrylamide gel concentration was 12% (Bio-Rad Laboratories, Vienna, Austria) instead of 14% (NuPAGE, Invitrogen Life Science, Carlsbad, CA, USA). The end-point titre of the sample used for reduction factor calculations was determined in a 0·5 $\log_{10}$ serial dilution setup and defined as the first dilution where no signal was observed on the Western blot. Samples were processed before PK digestion in order to overcome interference as detailed below.

Western blot validation

The Western blot assay used for determination of prion reduction factors and binding capacity in Tables 1 and 2 was subject to a formal validation following International Conference on Harmonisation (ICH) guidelines to enable an evaluation of the suitability of the assay in terms of assay variability and linearity for use in the clearance studies detailed in this report, and also an evaluation of the limit of detection (LOD) of the assay in comparison with a prion stock of known (defined) bioassay titre. The linearity of the assay is shown in Fig. 1. The regression parameters can be used to convert Western blot titres into infectious titres using the following formula:

$$\text{Titre}_{[\text{Bioassay}]} = \frac{\text{Titre}_{[\text{Western blot}]} + 4{\cdot}5867}{1{\cdot}0667}$$

This formula was used for calculation of the resin binding capacity in terms of infectious doses.

Table 1 PrP^{Sc} removal during chromatography with a non-S/D-treated spike. Fifty millilitres of Octaplas® final product was spiked at the indicated spike ratio with a CBH_{Sark} from hamsters infected with hamster-adapted scrapie 263 K strain. After withdrawal of a sample of the spiked start material, the spiked plasma was loaded onto the PRDT column and the flow-through fractions were collected. Following plasma loading and washing of the PRDT column with citrate buffer, the column was washed experimentally with 2 M NaCl, and finally the remaining resin was re-suspended in TBS and tested (column gel)

	Western blot 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

Table 2 PrP^{Sc} removal during Octaplas® manufacturing with an S/D-conditioned spike. Approximately 200 ml of crude plasma was spiked at a spike ratio of 1% with the indicated spike materials from hamsters infected with hamster-adapted scrapie 263 K strain. After withdrawal of a sample of the spiked start material, the spiked plasma was processed through a downscaled model of the Octaplas® process from front-end cell and cell-debris filtration, via S/D-treatment, filtration and solid phase extraction until eventually 50 ml of the S/D-treated plasma intermediate after solid phase extraction were loaded onto the 5 ml PRDT column from which the indicated flow-through fractions were collected. Following plasma loading and washing of the PRDT column with citrate buffer, the column was washed experimentally with 2 M NaCl, and finally the remaining resin was re-suspended in TBS and tested (column gel)

	Western blot 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 extraction and depth-filtration	2·5	1·0
After solid phase extraction	2·0	1·0
After 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

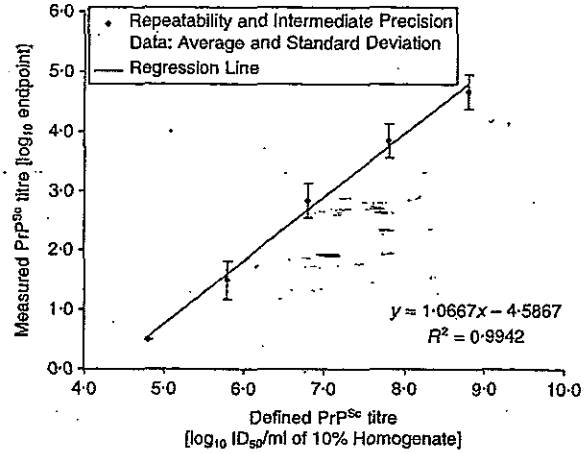

Fig. 1 Linearity of Western blot assay. A plot of Western blot end-point titres obtained from multiple determinations (at least 3) of various dilutions of a hamster-adapted scrapie 263 K prion stock of known (defined) bioassay titre. The limit of detection is 4·5 $log\ ID_{50}$/ml. The individual standard deviations for samples at each dilution tested was no greater than ±0·25 log_{10}.

Interference handling

A direct Western blotting of the samples containing Octaplas® could not be conducted due to the interference from high plasma protein content. To reduce this interference and to enable assaying of the flow-through samples after adsorption by the gel ligand, spiked samples were pre-diluted 3·2-fold (0·5 log_{10}) in TBS containing 0·1% bovine serum albumin followed by a centrifugation at 15 558 *g* for 60 min at ambient temperature. After centrifugation, the supernatant was carefully decanted and the pellet re-suspended in either the same volume of the original spiked sample, or in 1/10th the original volume centrifuged (i.e. 10-fold concentration), achieving an effective concentration of 0·5 log_{10}. Recovery within 0·5 log titre as determined by serial dilution Western blot assay of low titre PrP^{Sc} was demonstrated via this procedure in control experiments, as indicated by comparable Western blot end-point titres for the centrifuged samples when compared with a non-centrifuged sample (data not shown).

Regeneration samples containing basic high salt concentration were diluted 0·5 log_{10} and then tested in the Western blot assays. The column gel samples were tested undiluted before analysis by Western blotting (i.e. without centrifugation). The PK digestion was performed *in situ* on the matrix. Following boiling in SDS, the PrP^{Sc} was released from the matrix.

Robustness of the prion reduction step with regard to different spike preparations

Octaplas® was spiked with either MIC or $CBH_{[-MIC]}$ at a 1% spike ratio. The pH of the spiked material was determined and, if necessary, adjusted to a pH of 6·9–7·4.

Following removal of a sample for determination of titre in the spiked start material, the remaining spiked material was loaded onto a prepared ligand resin column (Vantage L11 X250, Millipore, Bedford, MA, USA), which had been equilibrated with water for injection, 20 mM citrate buffer, pH 7·0 containing 140 mM NaCl. The flow rate of the chromatography was adjusted to the necessary contact time (plasma with resin) of ~2 min. Collection of the flow-through began once the ultraviolet (UV) baseline had reached peak absorbance. Following loading of the sample, the column was washed with the citrate buffer used for equilibration, and collection of the flow-through continued until the UV absorbance began to drop. All chromatography steps were performed at ambient temperature. Samples (flow-through) were collected at various stages of the passage of the spiked start material through the column. An aliquot of each flow-through was stored at ≤ −60°C until tested by Western blotting as indicated above.

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

In order to evaluate the PrP^{Sc} binding capacity per millilitre gel, studies were performed using sequential identical columns. In these experiments, 0·01% CBH_{Sark} (final concentration of brain homogenate) was spiked in Octaplas® harvested from routine production. Ten millilitres of this challenge was applied to the first column (0·5 ml bed volume) containing the gel in a Protein Isolation Kit mini-column (PIKSI, ProMetic Life Sciences Inc., Mount Royal, Quebec, Canada). The flow-through from the first column was applied onto the second column – and from the second onto the third. The gel-bound PrP^{Sc} was quantified by densitometric reading of the Western blot signals, and the binding capacity per column and millilitre gel was estimated in comparison to the PrP^{Sc} input level.

Binding of infectious prions from different sources

Leucocyte-reduced human red blood cells in residual plasma spiked with brain homogenate from different transmissible spongiform encephalopathy strains, including hamster scrapie, human vCJD, human sporadic (sp)CJD, and mouse Fukuoka strain Gerstman–Sträussler–Scheinker disease (GSS), were applied in duplicate to the ligand resin in column format.

Calculation of reduction factors

Reduction factors (RF) were calculated as detailed in 'Note for Guidance on the Performance of Virus Clearance Studies' [CPMP/BWP/268/95 (1996)]: $RF = (V_1 \times T_1)/(V_2 \times T_2)$, in which V_1 and T_1 are the volume and titre of the start material – and V_2 and T_2 are the volume and titre of the product fraction, respectively. In logarithmic terms, this equation can be expressed as: $\log_{10}(RF) = [\log_{10}(V_1) + \log_{10}(T_1)] - [\log_{10}(V_2) + \log_{10}(T_2)]$, and the logarithmic reduction factors (LRF) were rounded to one decimal place only after having completed the final calculation.

Results

In preliminary studies, four of the most promising ligands among the many screened by the company PRDT [8,12] were selected for investigating their compatibility with the Octaplas® manufacturing process and its outcome. One of them did not change the biochemical profile of Octaplas® at all, whereas the other three depleted significantly both coagulation factors and inhibitors (data not shown).

Different aspects of prion binding were investigated by using different spike preparations. As unprocessed CBH probably contains all possible infectious modalities, it was used as the starting spike material for the various spike preparations. The MIC preparation has been chosen because it is enriched with the smallest and most soluble forms of PrP^{Sc}. Where the PrP^{Sc} concentration, as determined by Western blot, is theoretically unrelated to the size distribution of the prion aggregates, this spike with small PrP^{Sc} sizes may represent a form of infectivity closer to that assumed to be potentially present in plasma from blood donors than the form present in spikes with large particle sizes.

The CBH from which the microsomal fraction had been removed by centrifugation [$CBH_{(-MIC)}$] was selected to investigate the binding of larger particle size distributions, i.e. those not contained in the MIC fraction. The use of the two spike preparations above provides for a more thorough investigation of the binding properties of the ligand resin than when only CBH is used.

In addition, for some experiments a sarkosyl-solubilized spike was used. Sarkosyl-solubilized prion spike agents have been utilized widely in prion spiking studies, and yield a spike preparation from which the membrane components have been removed – which may mimic very well the nature of our target Octaplas® matrix following the S/D treatment. The use of sarkosyl as opposed to other detergents is a balance between avoiding extremely strong detergents, such as SDS, which may denature the prion aggregate, and using non-ionic detergents that tend to be too weak to provide sufficient solubilization. Where the spike material was solubilized with sarkosyl before spiking, the respective abbreviation for the spike material is appended with the subscripted text 'Sark' (i.e. CBH_{Sark}).

Feasibility experiments

In the first set of studies, experiments were performed where a sarkosyl-solubilized spike (i.e. lacking membrane components) was spiked into Octaplas® final product and applied directly onto PRDT columns. Two PRDT columns containing

the ligand resin at two different column volumes, 1·9 ml and 9·5 ml, were challenged with two concentrations of spiked Octaplas®, 1% and 5% spike ratios, respectively. The flow-through sample was collected in fractions as indicated in Table 1 and analysed by Western blot for PrP^{Sc}. Under a high PrP^{Sc} loading (i.e. 5% spike ratio), with 2·5 log_{10} as the input, a ≤ -0.5 log_{10} of PrP^{Sc} signal was recovered with a RF of ≥ 3.0 log_{10} (2·5 log_{10} minus ≤ -0.5 log_{10}) could be demonstrated for the early flow-through fractions (0–10 ml), utilizing the relevant Octaplas® matrix from manufacturing. We applied the methodology described above (see Interference handling) to remove the Western blot-interfering plasma proteins by assaying the pellet after centrifugation, which resulted in a quantitative recovery of the PrP^{Sc}. Furthermore, this centrifugation step provided 0·5 log_{10} of PrP^{Sc} concentration and, thus, increased the assay sensitivity. The results indicated that the binding capacity, determined by the volume at which breakthrough occurred, was dependent on the PrP^{Sc} load vs. the amount of affinity ligand in a reproducible manner. Within the accuracy of the assay, the total bound PrP^{Sc} loaded onto the column was quantitatively recovered – either in the experimentally applied 2 M NaCl wash or still bound to the gel.

PrP^{Sc} removal under manufacturing conditions

Further experiments were performed to investigate removal of PrP^{Sc} which had been conditioned via the S/D-treatment, filtration and solid phase extraction steps, which forms the mid-section of the standard Octaplas® manufacturing process. Crude plasma was spiked with hamster brain-derived infectivity and processed using a validated downscale of the manufacturing process, including the front-end cell and cell-debris filtration. Following the final solid phase extraction step, the product was loaded directly onto a PRDT column to investigate PrP^{Sc} removal. Note, that the level of removal observed for the Octaplas® manufacturing process before PRDT removal cannot be compared with that reported in previous publications which used a chronically infected whole cell preparation as spike. This earlier work measured prion removal for the Octaplas® process including cell removal via 1·0 μm filtration, whereas the current studies only addressed potential removal of non-cell associated prions post-1·0 μm filtration. Irrespective of the spike's nature [MIC or $CBH_{(-MIC)}$], an effective PrP^{Sc} removal to below the limit of assay sensitivity was observed in the early flow-through fractions from the column (Table 2). For the $CBH_{(-MIC)}$ spike, a slightly higher loss of spike material was observed for the steps before the column. Although not significant, this finding is consistent with the nature of this spike, which probably contained larger PrP^{Sc} aggregates or PrP^{Sc} associated with membranes fragments large enough to be filtered out. The pattern of breakthrough also demonstrates slight differences between the two spike materials, in which the MIC spike showed earlier breakthrough than the $CBH_{(-MIC)}$ spike. This result 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 may reflect the higher PrP^{Sc} loading onto the column due to the lower upstream loss of PrP^{Sc} compared to the $CBH_{(-MIC)}$ case. Again, for the early flow-through fractions (0–10 ml), the ≥ 3.0 log_{10} RF for the whole process (≥ 2.0–2·5 log_{10} RF by PRDT column) could be demonstrated using the MIC spike and the amount of PrP^{Sc} recovered from the experimental 2 M NaCl wash and gel demonstrate the substantial binding capacity of the affinity ligands. Based on the input of PrP^{Sc} and the sensitivity of the Western blot assay, it was calculated (see Materials and methods) that the PrP^{Sc} removal capacity per millilitre gel was 7·3 and 6·4 log_{10} 50% infectious dose (ID_{50})/ml resin for the MIC and $CBH_{(-MIC)}$ spike, respectively.

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

The gel binding capacity for PrP^{Sc} was also investigated utilizing a different study design, in which the PrP^{Sc} bound to the gel was analysed. In these studies, a fixed volume of challenge (10 ml) and a fixed volume of gel (0·5 ml) were used. The challenge concentration was 0·01% CBH_{Sark} (final concentration of brain homogenate). The spiked challenge solution was applied to three columns in series. The binding to each column was then evaluated independently via Western blotting. The results (Fig. 2) indicated that the vast majority of the detectable signal was concentrated in column 1. The flow-through from column 1 contained some contamination of PrP^{Sc}, which was visualized as a very weak signal captured by the second gel (< 3% of PrP^{Sc} input), as shown in Fig. 2. In all tests performed, no signal was ever detected in column 3 indicating that all PrP^{Sc} had been removed before this stage. Furthermore, this demonstrated very strong PrP^{Sc} capture was reproducible when different batches of gel were tested (Fig. 3). The quantification by densitometry of the PrP^{Sc} bands recovered from the resin was conducted using a Bio-Rad VersaDoc imaging system (Bio-Rad Laboratories, Hercules, CA, USA). The results indicated that practically all input PrP^{Sc} was detected bound to the resin. We had previously determined that the total ID_{50} in the challenge were 5×10^5 ID_{50} based on the infectivity titration of the spike with the bioassay. Thus, in all cases the PrP^{Sc} binding capacity per millilitre gel was found to be in the range of 5×10^5/0·5 ml gel, equivalent to 6·0 log_{10} ID_{50}/ml resin.

Determination of the gel ligand specificity for PrP^{Sc} from different sources

Figure 4 shows that the resin has the ability to bind infectious prion from all the sources tested, including the human vCJD

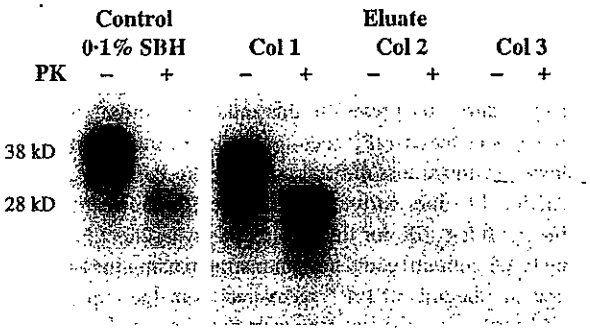

Fig. 2 Sequential PrP^{Sc} removal. Western blot analysis of the PrP^{Sc} protein eluted from the PRDT gel before (–) and after (+) PK treatment. The binding assay was conducted as described in Gregori *et al.* [8]. In brief, 10 ml of Octaplas® were spiked with 0·01% CBH_{Sark} (SBH) and applied to three columns (Col) in series, each column contained 0·5 ml of gel. In the –PK lanes, 50 µl of resin were mixed with 7·5 µl of water, 17·5 µl of 2% LDS and 25 µl of 4× LDS-sample buffer (NuPAGE). PK digestion was conducted directly on the gel beads (50 µl) with 7·5 µl of 1 mg/ml PK and 17·5 µl of 2% SDS incubated for 1 h with vigorous agitation. The reaction was stopped by the addition of 25 µl of 4× LDS-sample buffer (NuPAGE) containing the reducing agent. All samples were heated at 90 °C for 5 min, briefly centrifuged and 10 µl of the supernatant containing the eluted proteins were loaded on each lane. The control lanes show the PrP^{Sc} signal of 10 µl of 0·1% SBH before (–) and after (+) PK treatment. The PrP^{Sc} signal in the control lane (–PK) was used to estimate the amount of infectivity captured by the gel. The molecular weight standards in KDa are shown on the left.

and spCJD. In the case of spCJD, the signal was weak due to the low level of endogenous PrP^{Sc} in this particular specimen.

Discussion

A resin with a ligand, developed by the company PRDT, able to bind and remove PrP^{Sc} quickly and efficiently from plasma during the industrial manufacturing of the Octaplas® product has been identified. A number of studies have been performed investigating the clearance of PrP^{Sc} by this resin under a variety of conditions and utilizing various spike forms. The introduction of this prion binding step provides a robust and effective prion removal step dedicated to improving the prion safety profile of Octaplas® even further, without having a negative impact on the final product quality [13].

Various spike forms and study designs were used in order to evaluate the robustness of the PRDT resin. The resin challenged with CBH, detergent-soluble PrP^{Sc} forms, or homogenates enriched with small or large PrP^{Sc} forms all indicated several log-steps of consistent and reproducible removal ($\geq 3{\cdot}0$ $\log_{10}$). The PrP^{Sc} binding capacity of the resin per millilitre gel was shown to be in the region of 6·0–7·3 $\log_{10}$ ID_{50}/ml resin, and effective removal was observed up until the binding capacity of the column was reached. Thus, for the gel volume chosen (3·8 l) for a standard OctaplasLG®

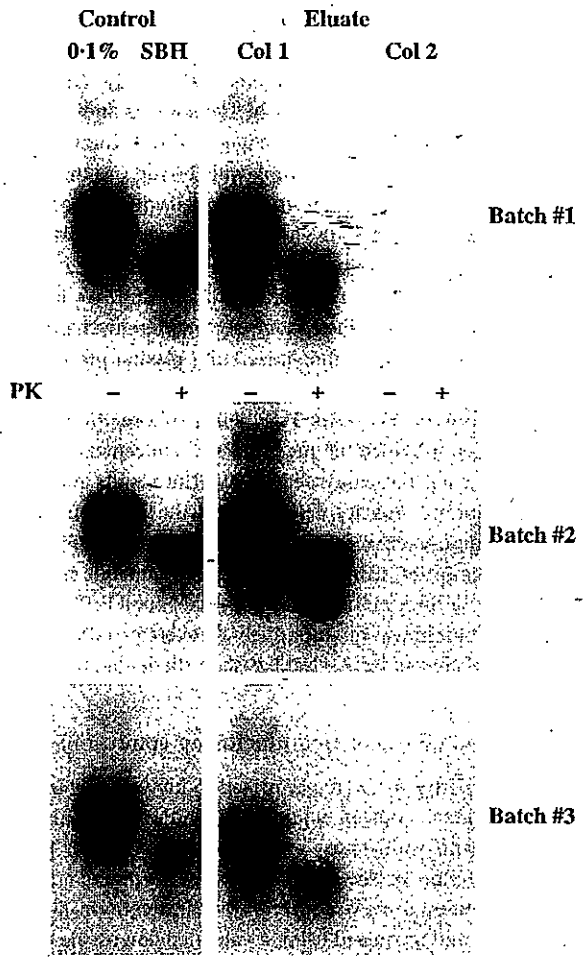

Fig. 3 Reproducibility of PrP^{Sc} removal in sequential set-up. Western blot comparison of PrP^{Sc} binding to three independently manufactured batches of PRDT gel. Ten millilitres of Octaplas® were spiked with 0·01% CBH_{Sark} (SHB) and applied to two columns (Col) in series, each column contained 0·5 ml of gel. The samples without (–) and with (+) PK were processed as described in Fig. 2. Ten microlitres of the eluted proteins were loaded on each lane. The control lanes show the PrP^{Sc} signal of 10 µl of 0·1% SHB before (–) and after (+) PK treatment.

batch size (380 l), 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 ($ID_{50} \times 0{\cdot}69$) [8]. In order to overload this removal capacity, every millilitre of such OctaplasLG® pools would need to contain more than 6900 ID PrP^{Sc}. Up to 20 ID/ml plasma have been found in relevant rodent models at the clinical stage of disease [14]. 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 OctaplasLG® pool, i.e. the gel capacity exceeds the prion load $\geq 218\,500$ times ($\geq 5{\cdot}3$ $\log_{10}$). Even with as many as 10 (1·6%) contaminated plasma units out of 630 plasmaphaeresis bags in an OctaplasLG® batch, the affinity ligand column is able to remove the total theoretical

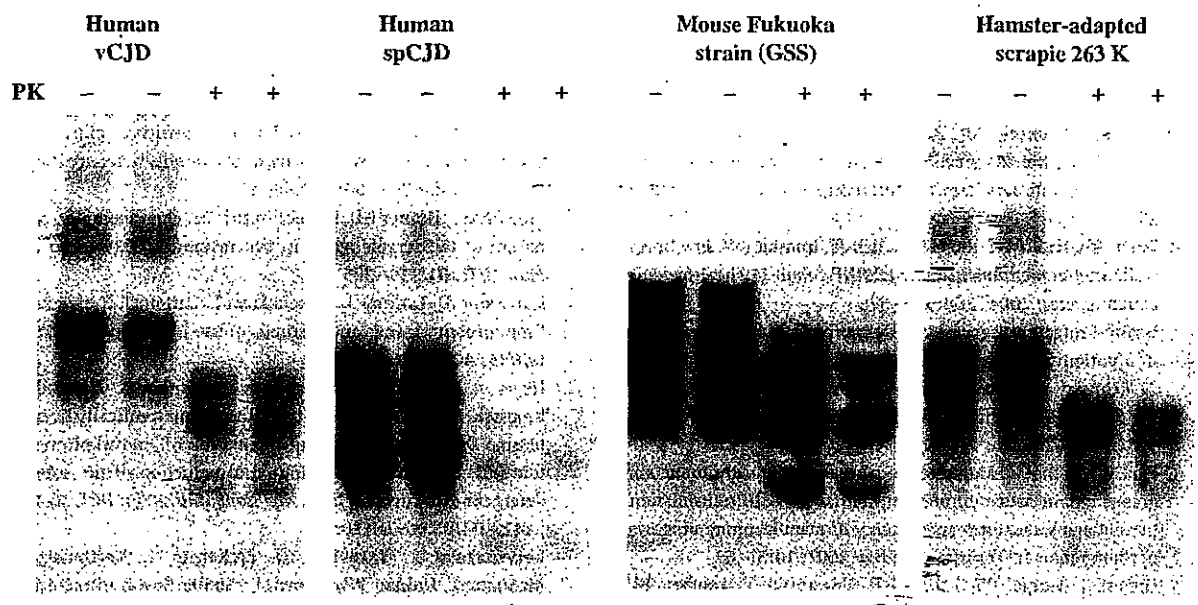

Fig. 4 Binding of PrP^{Sc} derived from various prion diseases. Western blot analysis of PrP^{Sc} binding to PRDT gel. Ten millilitres of human leukoreduced red blood cells in residual plasma were spiked with 1% CBH_{Sark} from a case of variant CJD (vCJD), a case of sporadic CJD (spCJD), a brain pool from mice infected with mouse-adapted Fukuoka strain (GSS) and 0·1% CBH_{Sark} pool from hamsters infected with hamster-adapted scrapie 263 K strain. Each sample was applied to 0·5 ml of resin in duplicate. Fifty microlitres of each resin [with (+) and without (–) PK treatment] were processed as described in Fig. 2. Ten microlitres of the eluted proteins were loaded on each lane. The exposure time of the film for each sample was adjusted to obtain equivalent signals intensity.

load of PrP^{Sc} with a safety margin higher than 21 850-fold ($\geq 4{\cdot}3$ $\log_{10}$). It is important to confirm the PrP^{Sc} binding demonstrated by Western blotting in these studies by animal infectivity studies. One such bioassay (hamsters) has just been completed successfully and the final result ($3{\cdot}0$ $\log_{10}$) confirmed the biochemical investigations summarized here (A. Bailey, personal communication). A second animal study is currently ongoing.

In theory, excessive amounts of PrP^{C} might be able to dislodge PrP^{Sc} that is already bound to the ligand in the gel. Thus, an experiment was performed to address this particular issue (data not shown). The normal concentration of PrP^{C} in plasma is estimated to be in the order of a few nanogram per millilitre of plasma [15,16]. 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. close to three orders of magnitude higher than the concentration normally found in plasma) to remove 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 OctaplasLG® batches would have no significant impact on the ability of the column to retain the gel-bound PrP^{Sc}.

In conclusion, the performed studies confirm a very effective PrP^{Sc} removal effect by the specific affinity ligand tested. The resin will be used in a chromatography step as a single-use resin, i.e. no sanitization and re-use. We have demonstrated that the introduction of the specific prion removal column into the current Octaplas® manufacturing process is technologically possible and will further improve the safety margin of this product in terms of prion diseases such as vCJD. The new generation Octaplas® will be marketed as OctaplasLG®.

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B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料３において集積報告を行っているため、添付していない）。