

Biochemical quality of the pharmaceutically licensed plasma OctaplasLG[®] after implementation of a novel prion protein (PrP^{Sc}) removal technology and reduction of the solvent/detergent (S/D) process time

A. Heger,¹ T.-E. Svae,¹ A. Neisser-Svae, S. Jordan,² M. Behizad² & J. Römisch¹

¹Research and Development Department, Octapharma Pharmazeutika Produktionsges.mbH, Vienna, Austria

²ProMetic BioSciences Ltd., Cambridge Science Park, Cambridge, UK

Vox Sanguinis

Background and Objectives A new chromatographic step for the selective binding of pathological prion proteins (PrP^{Sc}) to an affinity ligand, developed and optimized for PrP^{Sc} capture and attached to synthetic resin particles (PRDT, USA; ProMetic BioSciences Ltd, Isle of Man, UK) was implemented into the manufacturing process of the solvent/detergent (S/D) treated biopharmaceutical quality plasma Octaplas[®].

Materials and Methods Pilot batches of Octaplas[®] with the implemented chromatographic step [labelled as OctaplasLG[®] (ligand gel)] were manufactured by Octapharma PPGmbH, Vienna, Austria. The biochemical quality was compared directly after manufacturing as well as after 18 months storage. All samples were tested on global coagulation parameters, fibrinogen levels, activities of coagulation factors and protease inhibitors, ADAMTS13 levels, as well as markers of activated coagulation and fibrinolysis. In addition, von Willebrand factor multimeric analysis was performed.

Results The incorporation of this novel chromatography into the large-scale routine manufacturing process was shown to be technically feasible and the performance of the column was assessed to be excellent. The biochemical studies showed that Octaplas[®] and OctaplasLG[®] produced without and with the new column, respectively, demonstrate an identical biochemical quality. OctaplasLG[®] remained stable over a period of 18 months stored frozen. A parallel reduction of the S/D virus inactivation step from 4–4.5 to 1–1.5 h led to significantly higher activities of plasmin inhibitor.

Conclusion The studies confirmed that the affinity ligand chromatography under the developed conditions can be introduced into the Octaplas[®] manufacturing process, as a mean to reduce potentially present PrP^{Sc}, without hampering the proven quality of this product.

Key words: Octaplas[®], OctaplasLG[®], biochemical characterization, plasma quality, affinity ligand chromatography, PrP^{Sc}.

Received: 28 November 2008,

revised 16 March 2009,

accepted 17 March 2009,

published online 9 April 2009

Correspondence: Dr Andrea Heger, Octapharma Pharmazeutika Produktionsges.m.b.H., Oberlaaer Strasse 235, A-1100 Vienna, Austria.

E-mail: andrea.heger@octapharma.com

Introduction

Variant Creutzfeldt–Jakob disease (vCJD) is a novel human prion disease caused by the infectious agent of bovine spongiform encephalopathy (BSE) [1]. Although the vCJD agent has not been detected in plasma from clinical cases of the disease [2,3], all manufacturers of plasma or plasma-derivatives have been encouraged by health authorities worldwide to investigate the potential of their manufacturing processes to remove or inactivate prions and/or introduce new prion clearance technologies [4]. Octaplas® is a solvent/detergent (S/D) treated, pharmaceutically licensed, alternative to single-donor fresh-frozen plasma (FFP) for transfusion [5–8]. The evaluation of the current Octaplas® manufacturing process to remove potentially present pathologic prion proteins (PrP^{Sc}) revealed an overall capacity of 2.5 log₁₀ [9]. A further extension of this removal capacity has been evaluated by investigating a number of other options, using in particular depth-filters and different nanofilters. None of these options met the required combination of a significant PrP^{Sc} removal capacity and technical feasibility without hampering significantly the biochemical quality of Octaplas®. As an alternative, selective adsorption of PrP^{Sc} to an affinity ligand optimized for prion protein capture and attached to synthetic resin particles [developed by the company PRDT (Pathogen Removal and Diagnostic Technologies Inc., USA), and manufactured by ProMetic BioSciences Ltd, Isle of Man, UK] was investigated, revealing promising results in a number of laboratory-scale biochemistry experiments and prion validation studies.

The implementation of novel technologies into manufacturing processes may have an impact on the quality of plasma-derived products. Therefore, the aim of our studies was not only to evaluate the technical performance of the incorporated chromatographic step, but also to demonstrate that the quality of Octaplas® is not impaired by the introduction of this new technology at large-scale routine manufacturing. Until now, six batches of Octaplas® with implemented affinity ligand chromatography step for the specific capture of PrP^{Sc} were produced (the product was labelled as OctaplasLG®, i.e. ligand gel), and an extensive biochemical characterization of these batches was performed. Final containers of normal Octaplas® produced without the additional prion protein removal step were used as control samples. All plasma samples were tested on screening tests of blood coagulation, fibrinogen levels, the activities of coagulation factors and inhibitors of blood coagulation and fibrinolysis, as well as markers of activated coagulation and fibrinolysis. Furthermore, thrombin generation assay (TGA) and thromboelastography (TEG) were used as functional assays. Finally, von Willebrand factor (VWF) multimeric analyses were performed. The results of the extensive biochemical characterization of

six OctaplasLG® pilot batches with implemented ligand chromatography for PrP^{Sc} capture are reported and discussed in details.

Materials and methods

Materials

Six batches OctaplasLG® were produced by Octapharma PPGmbH (Vienna, Austria) under production conditions in 2007 and 2008. The affinity ligand chromatography using synthetic resin particles with ligands for selective adsorption of PrP^{Sc}, manufactured by ProMetic BioSciences Ltd., was introduced into the large-scale Octaplas® manufacturing process after complete cell removal and S/D treatment. Batches 1–3, labelled as OctaplasLG®/1, were produced with 4–4.5 h S/D virus inactivation step, according to the current Octaplas® manufacturing process. For the batches 4–6, labelled as OctaplasLG®/2, parallel to the implementation of the prion protein removal step, the S/D virus inactivation was reduced from 4–4.5 to 1–1.5 h. Final bags of normal Octaplas® produced without the additional prion protein removal step were used as control samples. Prior to testing, the plasma samples were thawed in a water bath at +37°C according to standard operating procedures.

Analytical assays

Visual control, haemagglutinins, pH, sodium, potassium, calcium, citrate, phosphate, glycine, osmolality, tri-(n-butyl)-phosphate (TNBP), octoxynol-9, irregular erythrocyte antibodies, sterility, pyrogens, as well as anti-hepatitis A virus (HAV) were assessed according to European Pharmacopoeia methods. Protein composition was determined by electrophoresis and total protein by the Biuret method. Anti-parvovirus B19 IgG was measured by ELISA. TGA was performed using the Technothrombin TGA test kit from Technoclone GmbH (Vienna, Austria). TEG measurements were performed by ROTEG-thromboelastography (Pentapharm GmbH, Munich, Germany). From 100 µl of whole blood centrifuged 10 min at 3000 × *g*, plasma was removed and replaced with 300 µl of plasma samples. Three hundred microlitres of this blood reconstituted with plasma was recalcified with 20 µl StaTEG-reagent and clotting parameters [e.g. clotting time (CT), clot formation time (CFT)] were analysed. ELISA test kits were used for the quantitative determination of the ADAMTS13 activity and antigen levels (Technozym ADAMTS13 ELISA, Technoclone GmbH), prothrombin split products (F1+2; Enzygnost F1+2, monoclonal, Siemens Healthcare Diagnostics), thrombin-antithrombin complexes (TAT, Enzygnost TAT micro, Siemens Healthcare Diagnostics) and D-dimer levels (Asserachrom D-Dimer, Diagnostica Stago). VWF multimeric analyses were performed using 1.2% agarose gel electrophoresis

Table 1 Quality control assays

Parameters	European Pharmacopoeia 6:2 07/2008:1646	Octaplas® final product specification	Octaplas® (n = 3)	OctaplasLG®/1 (n = 3)	OctaplasLG®/2 (n = 3)
Visual control	a	a	Passed	Passed	Passed
Protein composition	b	b	Passed	Passed	Passed
Haemagglutinins	c	c	Passed	Passed	Passed
Total protein (mg/ml)	≥ 45	45–70	62 (61–62)	61 (60–63)	58 (57–58)
pH value	6.5–7.6	7.0–7.6	7.2 (7.2–7.3)	7.5 (7.4–7.5)	7.3 (7.3–7.4)
Sodium (mmol/l)	≤ 200	≤ 200	159 (154–168)	159 (155–165)	161 (157–163)
Potassium (mmol/l)	≤ 5.0	≤ 5.0	3.6 (3.5–3.8)	3.7 (3.6–3.7)	3.6 (3.5–3.7)
Calcium (mmol/l)	≤ 5.0	≤ 5.0	2.0 (2.0–2.1)	2.0 (2.0–2.0)	2.0 (1.9–2.0)
Citrate (mmol/l)	≤ 25	15–25	19 (18–21)	20 (18–22)	22 (21–22)
Phosphate (mmol/l)	NS	2.0–7.5	5.3 (4.5–6.3)	5.2 (4.4–6.2)	6.2 (5.8–6.8)
Glycine (mg/ml)	NS	4.0–6.0	5.1 (5.0–5.2)	5.1 (5.1–5.2)	5.0 (4.7–5.3)
Osmolality (mOsmol/kg)	≥ 240	320–420	359 (354–364)	357 (351–364)	364 (361–370)
Tri-(n-butyl)-phosphate (μg/ml)	< 2	≤ 2.0	< 0.5	< 0.5	< 0.5
Octoxynol-9 (μg/ml)	< 5	≤ 5.0	≤ 2.3	≤ 2.0	≤ 0.7
Fibrinogen (mg/ml)	NS	1.5–4.0	2.7 (2.6–2.8)	2.6 (2.6–2.7)	2.6 (2.5–2.7)
Irregular erythrocyte antibodies	d	d	Passed	Passed	Passed
Sterility	e	e	Passed	Passed	Passed
Pyrogens	f	f	Passed	Passed	Passed
Anti-hepatitis A virus IgG (IU/ml)	≥ 1	≥ 1	2 (1–2)	2 (1–2)	2 (2–3)
Anti-Parvo B19 IgG (IU/ml)	NS	≥ 20	34 (33–36)	32 (31–34)	37 (36–40)
Factor V (IU/ml)	≥ 0.5	≥ 0.5	0.8 (0.8–0.9)	0.8 (0.8–0.9)	0.8 (0.7–0.8)
Factor VIII (IU/ml)	≥ 0.5	≥ 0.5	0.8 (0.6–0.9)	0.7 (0.5–0.9)	0.8 (0.7–0.8)
Factor XI (IU/ml)	≥ 0.5	≥ 0.5	0.8 (0.7–0.8)	0.8 (0.8–0.8)	0.9 (0.8–0.9)
Activated partial thromboplastin time (sec)	NS	23–40	29 (29–30)	29 (29–30)	30 (29–31)

Mean (minimum–maximum) levels are presented; NS, not specified.

^aClear or slightly opalescent, free of solid or gelatinous particles; ^bCorresponds to the pattern of normal human plasma; ^cCorresponds to blood group stated on the label; ^dNo irregular erythrocyte antibodies; ^eSterile; ^fFree of pyrogens.

according to a modification of the method described by Metzner *et al.* [8,10]. All other parameters were tested by commercially available test kits as described earlier [7,8]. The Student's paired *t*-test was used to assess statistically significant differences between the quality of Octaplas® and OctaplasLG® pilot batches. A *P*-value of < 0.05 was considered as statistically significant.

Results

Technical performance of the affinity ligand chromatography

The affinity ligand chromatography for specific PrP^{Sc} capture was incorporated into the Octaplas® manufacturing process after the complete cell removal and S/D treatment, representing a stage at which cells and debris that might harbour PrP^{Sc} have been fully removed (i.e. clear, liquid matrix). The technical performance of the implemented affinity ligand chromatography was very good in the industrial set-up

developed. The flow-rate, ensuring the correct contact time between the plasma matrix and affinity ligand gel, could be well controlled. The integrity of the column was fully maintained, i.e. no channelling. Due to the reduction of the S/D virus inactivation time, there was no major prolongation of the total production time.

Biochemical characterization of OctaplasLG®

After thawing, all six batches of OctaplasLG® were clear and free of solid or gelatinous particles, the protein composition corresponded to the pattern of normal human plasma, and haemagglutinins corresponded to blood groups stated on the label (Table 1). Both pH levels and osmolality remained within the approved specification for Octaplas® (i.e. 7.0–7.6 and 320–420 mOsmol/kg, respectively). The total protein and fibrinogen levels were in the ranges 57–63 and 2.5–2.7 mg/ml, respectively, i.e. within the normal ranges found in plasma. Factor V, VIII and XI activities were 0.5 IU/ml or higher; the lowest factor VIII activity of 0.5 IU/ml was found in a blood

Table 2 Additional assays

Parameters	Octaplas® (n = 3)	OctaplasLG®/1 (n = 3)	OctaplasLG®/2 (n = 3)
Prothrombin time (sec)	10.5 (9.9–10.9)	10.4 (9.8–10.9)	10.9 (10.5–11.5)
Reptilase time (sec)	20.3 (18.4–23.4)	19.6 (18.7–20.7)	17.4 (17.1–17.6)
Thrombin time (sec)	14.7 (13.2–15.8)	14.5 (13.4–15.3)	14.4 (14.2–14.7)
Factor II (IU/ml)	0.98 (0.94–1.04)	0.94 (0.92–0.97)	1.01 (0.90–1.08)
Factor VII (IU/ml)	0.93 (0.83–1.05)	0.90 (0.83–0.93)	1.10 (1.03–1.16)
Factor IX (IU/ml)	0.84 (0.78–0.94)	0.83 (0.75–0.87)	0.85 (0.77–0.92)
Factor X (IU/ml)	1.03 (0.93–1.15)	1.01 (0.91–1.10)	0.99 (0.91–1.04)
Factor XII (IU/ml)	0.96 (0.92–0.98)	0.91 (0.87–0.94)	1.05 (0.92–1.11)
Factor XIII (IU/ml)	1.00 (0.93–1.10)	0.94 (0.88–0.99)	1.02 (0.97–1.10)
von Willebrand factor:RCo (IU/ml)	0.95 (0.86–1.10)	1.00 (0.99–1.00)	0.89 (0.84–1.00)
ADAMTS13 activity (IU/ml)	0.91 (0.81–1.10)	0.92 (0.82–1.00)	1.01 (0.96–1.10)
ADAMTS13 antigen (IU/ml)	0.99 (0.93–1.05)	1.00 (0.96–1.03)	1.03 (0.98–1.10)
Antithrombin (IU/ml)	0.92 (0.86–1.01)	0.95 (0.81–1.07)	0.94 (0.92–0.98)
Heparin cofactor II (IU/ml)	0.95 (0.92–0.96)	0.96 (0.95–0.96)	1.12 (1.05–1.16)
Protein C (IU/ml)	0.93 (0.91–0.94)	0.93 (0.92–0.93)	0.86 (0.79–0.98)
Protein S (IU/ml)	0.66 (0.65–0.66)	0.55 (0.53–0.58)	0.66 (0.61–0.72)
Plasmin inhibitor (IU/ml)	0.33 (0.30–0.35)	0.33 (0.31–0.35) ^a	0.63 (0.61–0.64) ^a
α ₁ -Antitrypsin (mg/ml)	0.99 (0.93–1.05)	0.99 (0.88–1.10)	1.05 (0.95–1.15)
C1-inhibitor (IU/ml)	0.61 (0.60–0.63)	0.59 (0.59–0.60)	0.72 (0.69–0.74)
Albumin (mg/ml)	31.4 (30.3–32.6)	31.6 (30.0–32.6)	29.1 (28.5–29.6)
IgG (mg/ml)	7.5 (7.2–8.0)	7.5 (6.8–8.0)	8.1 (6.9–8.7)
IgA (mg/ml)	1.5 (1.3–1.6)	1.4 (1.3–1.4)	1.6 (1.5–1.7)
IgM (mg/ml)	0.66 (0.64–0.68)	0.69 (0.65–0.73)	0.81 (0.80–0.82)
Lipoprotein (a) (mg/dL)	8.0 (5.2–9.5)	6.7 (5.4–7.5)	9.1 (8.6–9.8)
Plasminogen (IU/ml)	0.93 (0.82–1.09)	0.94 (0.90–1.03)	0.83 (0.75–0.91)
C3 (mg/dL)	101 (98–103)	100 (95–105)	119 (110–124)
C4 (mg/dL)	18 (14–21)	18 (16–19)	19 (16–23)
Factor VIIa (mIU/ml)	83 (80–88)	86 (81–90)	94 (86–102)
TAT (μg/l)	1.6 (1.0–2.2)	2.0 (1.7–2.5)	2.4 (2.2–2.5)
F1+2 (nmol/l)	0.18 (0.15–0.20)	0.20 (0.17–0.22)	0.15 (0.15–0.15)
D-dimer (ng/ml)	132 (117–142)	138 (122–158)	114 (112–116)

Mean (minimum–maximum) levels are presented; ^aStatistically significant difference between OctaplasLG®/1 and OctaplasLG®/2 batches (i.e. *P*-value < 0.05).

group O batch. The levels of activated partial thromboplastin time (aPTT) were within the Octaplas® specification (i.e. 23–40 s). There were no significant differences observed in the sodium, potassium, calcium, citrate, phosphate and glycine levels between Octaplas® and OctaplasLG® final products. TNBP and octoxynol-9 levels were within the specification levels, mainly below the limits of detection. In addition, no irregular erythrocyte antibodies were found, both products were sterile and free of pyrogens. Anti-parvovirus B19 IgG levels were higher than 20 IU/ml in all bags, whereas anti-HAV IgG varied between 1 and 3 IU/ml.

There were no significant differences in global coagulation parameters obtained in Octaplas® and OctaplasLG® final products (Table 2). No prolongation of the prothrombin time (PT) and aPTT was observed, indicating no relevant depletion

of coagulation factors required for both the extrinsic and the intrinsic pathway of coagulation. In addition, there was no prolongation of the thrombin time (TT) and reptilase time (RT) by decreased or dysfunctional fibrinogen observed. The thrombin generation potential was assessed and compared, reflecting a composite effect of the multiple factors determining coagulation capacity (Fig. 1/1). There were no significant differences in TGA parameters tested in Octaplas® produced with and without the ligand chromatography. All thrombin concentrations (393 to 439 nm) were within the reference ranges indicated in the lot-specific batch table of the Technoclone test kit, i.e. 355–591 nm. TEG, upon supplementation of sufficient surfaces to drive coagulation by freshly prepared whole blood cells of healthy subjects, was performed to investigate the dynamics of blood coagulation, clot formation

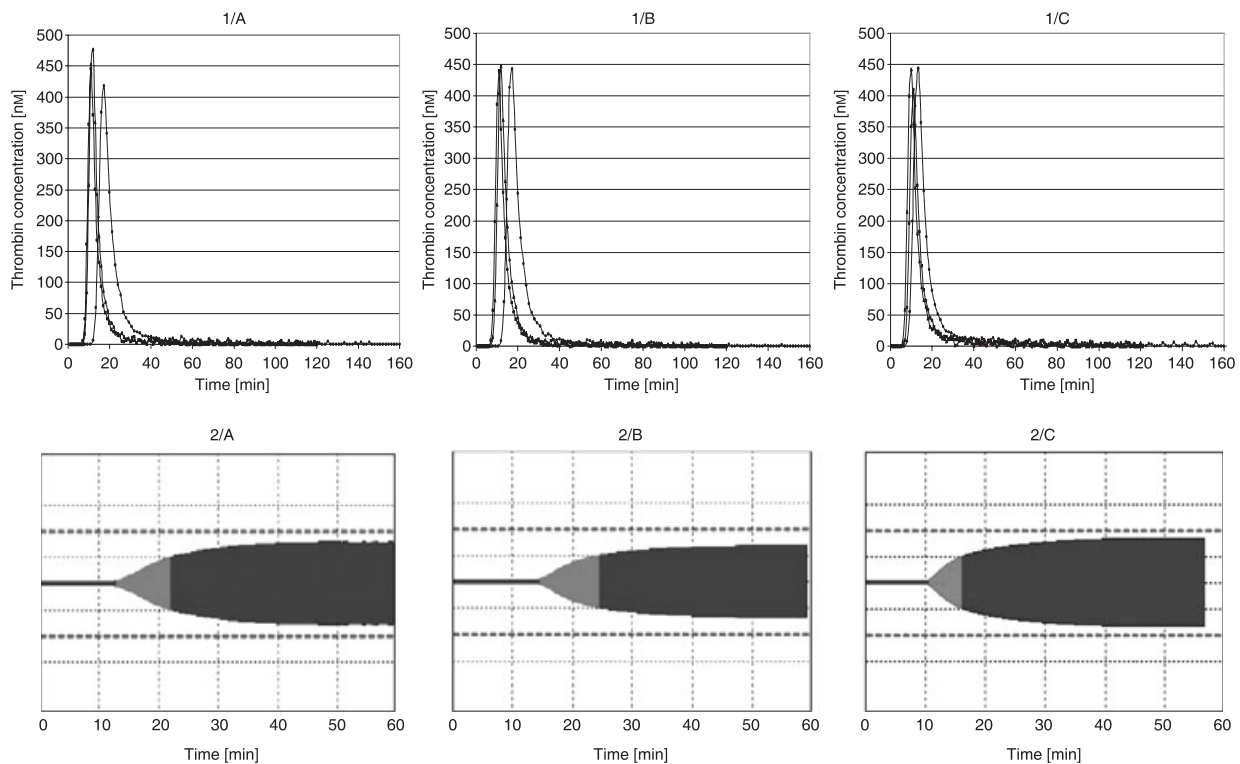


Fig. 1 Determination of Octaplas[®] samples using thrombin generation assay (TGA) (1) and thromboelastography (TEG) (2) functional assay. Results for Octaplas[®] (A), OctaplasLG[®]/1 (B) and OctaplasLG[®]/2 (C) are presented. For TEG, results for one representative batch are shown.

and clot characteristics (Fig. 1/2). Recalcification TEG showed no significant differences in Octaplas[®] produced with and without affinity ligand chromatography. Mean levels of the clotting parameters in OctaplasLG[®] batches were within the reference range indicated in the test kit (300–1000 s for CT and 150–700 s for CFT, respectively).

Coagulation factor activities in all six batches of OctaplasLG[®] were within both the normal ranges for single-donor FFP and the valid Octaplas[®] product specification (i.e. ≥ 0.5 IU/ml, Table 2). In addition, there were no significant differences in both ADAMTS13 activity and antigen levels between the different product groups (Table 2). From the protease inhibitors tested, the activities of antithrombin, heparin cofactor II, protein C, α_1 -antitrypsin and C1-esterase inhibitor were within the normal ranges for single-donor FFP (Table 2). No significant differences in activity levels between Octaplas[®] and OctaplasLG[®] batches were observed. As a result of the standard S/D treatment, protein S and plasmin inhibitor activities were decreased in Octaplas[®] to mean levels of 0.66 IU/ml and 0.33 IU/ml, respectively. The mean protein S and plasmin inhibitor activities in the OctaplasLG[®]/1 and OctaplasLG[®]/2 final products were 0.55/0.66 and 0.33/0.63 IU/ml, respectively. Statistically significant differences ($P < 0.05$) in the plasmin inhibitor activities of the different OctaplasLG[®] batches were due to the differences in the duration of the S/D virus

inactivation step. In OctaplasLG[®]/2 batches produced by 1–1.5 h S/D treatment, plasmin inhibitor activities were significantly higher (mean 0.63 IU/ml) than in OctaplasLG[®]/1 (mean 0.33 IU/ml).

The contents of a selected number of major plasma proteins, comprising albumin, IgG, IgA, IgM and lipoprotein (a), were within the normal ranges for plasma in all samples tested (Table 2). In addition, both components of the fibrinolytic (i.e. plasminogen) and complement system (i.e. C3 and C4) were found in comparable concentrations in Octaplas[®], OctaplasLG[®]/1 and OctaplasLG[®]/2, respectively (Table 2). Finally, indicators of coagulation activation and fibrinolysis were tested (Table 2). FVIIa levels in OctaplasLG[®] ranged between 81 and 102 mIU/ml, representing physiological plasma concentrations indicating that there was no activation of FVII during the implemented ligand chromatography step. TAT levels (result of low level thrombin generation and subsequent inhibition by antithrombin), F1+2 levels (indicator of traces of prothrombin activation) and D-dimer levels (released by plasmin action towards formed fibrin) in all OctaplasLG[®] batches were within the normal ranges (Table 2). There were no significant differences in the levels of indicators of coagulation activation and fibrinolysis between Octaplas[®] batches produced with and without ligand chromatography, as well as OctaplasLG[®] produced with 4–4.5 and 1–1.5 h S/D

virus inactivation (i.e. OctaplasLG[®]/1 and OctaplasLG[®]/2, respectively).

The VWF multimeric patterns, obtained by high-resolution 1.2% agarose gel electrophoresis, were comparable between the Octaplas[®] samples produced with and without affinity ligand chromatography (data not shown, see [8]). There were no ultralarge molecular weight forms of VWF multimers observed in any of the plasma samples tested. Furthermore, the triplet structure of VWF was found to be normal, i.e. not subjected to proteolytic cleavage.

Stability studies on OctaplasLG[®]

A long-term stability study of the pilot batches OctaplasLG[®] is ongoing at $\leq -30^{\circ}\text{C}$ and $-18^{\circ}\text{C} \pm 2^{\circ}\text{C}$. At the time, results are available after 3, 6, 9, 12 and 18 months storage of OctaplasLG[®]/1 as well as after 3 months storage of OctaplasLG[®]/2 at both temperatures. There were no significant differences in the activities of coagulation factors and protease inhibitors at any temperature and time point, and all activities varied within the biopharmaceutical range of $\pm 20\%$ and in the normal ranges for S/D plasma. There were no significant prolongations of the aPTT, PT, RT and TT observed after storage at any temperature. No activation of parameters of blood coagulation and fibrinolysis occurred.

Discussion

The residual amount of blood cells in blood and blood plasma is a possible risk factor for cell-associated adverse reactions and pathogen transmission, including diseases associated with prion infectivity, i.e. vCJD [11–13]. Octaplas[®] is produced from up to 1520 leucocyte-reduced, single-donor FFP units. The manufacturing process includes several filtration steps, including $1.0\ \mu\text{m}$ size-exclusion filtration, depth filtration and sterile filtration, leading to a completely cell-free plasma product [14]. The evaluation of the current Octaplas[®] manufacturing process to remove cell-bound and cell-free PrP^{Sc} revealed an overall capacity of $2.5\ \log_{10}$ [9]. While the infectious agent of vCJD is very resistant against the virus inactivation techniques such as the S/D method [15], a number of other methods were evaluated to further enhance the PrP^{Sc} removal capacity of this plasma product. Technologies commonly used for harvesting and purification of plasma derivatives, such as protein precipitations/extractions, column harvesting/purifications and filtration techniques, with high efficacy to remove/inactivate PrP^{Sc} [16], cannot be introduced into the manufacturing process without hampering significantly the biochemical quality of Octaplas[®]. From a panel of affinity ligands, derived from screening large solid-phase combinatorial libraries (by the company PRDT), four constructs were selected for extensive studies to assess prion protein capture in laboratory-scale laboratory experiments and prion validation studies.

All four immobilized ligands (attached to synthetic resin particles) were able to bind exogenous PrP^{Sc} infectivity in plasma at high capacity [17], however, three of the four ligands tested affected the Octaplas[®] quality significantly (data not shown). Results obtained for the fourth ligand showed that the implementation of the chromatographic step with this ligand into the Octaplas[®] manufacturing step is feasible both from a biochemical quality and pathogen safety point of view and resulted in a process reduction of $\geq 3.0\ \log_{10}$ PrP^{Sc} (Neisser-Svae *et al.*, unpublished data).

Six large-scale batches of OctaplasLG[®] with implemented affinity ligand chromatography step for the removal of the PrP^{Sc} were manufactured. To allow maximum binding of PrP^{Sc}, the column was incorporated into the manufacturing process after complete cell removal and S/D treatment. The flow-rate, ensuring the correct contact time between the plasma matrix and the affinity ligand gel, was well controlled in the industrial set-up developed. In addition, the performance of the column was at full good manufacturing practice (GMP) level. Due to the parallel reduction of the S/D virus inactivation time, the overall production time for Octaplas[®] remained unchanged. Validation studies demonstrated that the S/D method inactivates enveloped viruses within a few minutes to below the limit of detection [18]). Therefore, the process time of 1–1.5 h is more than enough to ensure the viral safety of OctaplasLG[®] towards enveloped viruses and provides a large safety margin for viruses such as HIV, HBV, HCV and WNV. Extensive biochemical characterization studies showed that Octaplas[®] final products produced with and without the implemented affinity ligand chromatography demonstrate an identical plasma quality. The quality of the product could be further improved by reduction of the S/D virus inactivation time from 4–4.5 to 1–1.5 h. The 1–1.5 h S/D inactivation in the presence of 1% TNBP and 1% octoxynol-9 resulted in significantly higher plasmin inhibitor activities and maintained levels of protein S in OctaplasLG[®]/2 (approximately 0.6 IU/ml for both protease inhibitors), while maintaining the same virus safety level. In addition, extensive stability studies showed no significant changes in the plasma quality of OctaplasLG[®]/1 after 18 months storage at both $\leq -30^{\circ}\text{C}$ and $-18^{\circ}\text{C} \pm 2^{\circ}\text{C}$.

In conclusion, the above studies confirmed that affinity ligand chromatography under the described chromatographic conditions can be introduced into the Octaplas[®] manufacturing process as a mean to reduce potentially present PrP^{Sc}. Based on the comprehensive biochemical investigation and stability studies performed, it must be expected that there is no difference in clinical safety and efficacy of OctaplasLG[®] (based on the production approach described for OctaplasLG[®]/2) compared to that demonstrated by Octaplas[®] over the last 17 years, except for the increased safety margin in terms of prion disease transmission and the possible effect of a significantly increased plasmin inhibitor activity.

Acknowledgements

The authors thank Bettina Prager at Research & Development, as well as Michael Tschannerl and Thomas Eiter at Octaplas® Production Department, Octapharma PPGmbH, for their scientific guidance during the optimization and/or upscaling of the affinity ligand chromatography. We thank the laboratory staff of Octapharma's Research & Development and Quality Control Department for the excellent analytical assay work. Additional thank goes to Jennifer Walden and Anna Reeves from ProMetic BioSciences Ltd. for their outstanding scientific work during the resin validation studies. Finally, we gratefully acknowledge the excellent collaboration with Peter Edwardson and Steve Burton at ProMetic BioSciences Ltd.

References

- 1 Will RG, Ironside JW, Zeidler M, Cousens SN, Estibeiro K, Alperovitch A, Poser S, Pocchiari M, Hofman A, Smith PG: A new variant of Creutzfeldt–Jakob disease in the UK. *Lancet* 1996; **347**:921–925
- 2 Wadsworth JDF, Jonier F, Hill AF, Campbell TA, Desbruslais M, Luthert PJ, Collinge J: Tissue distribution of protease resistant prion protein in variant Creutzfeldt–Jakob disease using a highly sensitive immunoblotting assay. *Lancet* 2001; **358**:171–180
- 3 Bruce ME, McConnell Will RG, Ironside JW: Detection of variant Creutzfeldt–Jakob disease infectivity in extraneural tissues. *Lancet* 2001; **358**:208–209
- 4 EMEA/CPMB/BWP/2879/02/rev 1. CHMP Position statement on Creutzfeldt–Jakob disease and plasma-derived and urine-derived medicinal products. June 2004
- 5 Hellstern P, Sachse H, Schwinn H, Oberfrank K: Manufacture and *in vitro* characterization of solvent/detergent-treated human plasma. *Vox Sang* 1992; **63**:178–185
- 6 Beeck H, Hellstern P: *In vitro* characterization of solvent/detergent-treated human plasma and of quarantine fresh frozen plasma. *Vox Sang* 1998; **74**:219–223
- 7 Heger A, Römisch J, Svae TE: A biochemical comparison of a pharmaceutically licensed coagulation active plasma (Octaplas) with a universally applicable development product (Uniplas) and single-donor FFPs subjected to methylene-blue dye and white-light treatment. *Transfusion Apher Sci* 2006; **35**:223–233
- 8 Heger A, Kannicht C, Römisch J, Svae TE: Normal levels of ADAMTS13 and factor H are present in the pharmaceutically licensed plasma for transfusion (Octaplas) and in the universally applicable plasma (Uniplas) in development. *Vox Sang* 2007; **92**:206–212
- 9 Svae TE, Neisser-Svae A, Bailey A, Reichl H, Biesert L, Schmidt T, Heger A, Römisch J: Prion safety of transfusion plasma and plasma-derivatives typically used for prophylactic treatment. *Transfusion Apher Sci* 2008; **39**:59–67
- 10 Metzner HJ, Hermentin P, Cuesta-Linker T, Langner S, Müller HG, Friedebold J: Characterization of factor VIII/von Willebrand factor concentrates using a modified method of von Willebrand multimer analysis. *Haemophilia* 1998; **4**:25–32
- 11 Brown P, Rohwer RG, Dunstan BC, MacAuley C, Gajdusek DC, Drohan WN: The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathies. *Transfusion* 1998; **38**:810–816
- 12 Williamson LM: New-variant Creutzfeldt–Jakob disease and leukocyte depletion of blood components. *Infusionsther Transfusionsmed* 1999; **26**:24–30
- 13 Gregori L, McCombie N, Palmer D, Birch P, Sowemimo-Coker SO, Giulivi A, Rohwer RG: Effectiveness of leucoreduction for removal of infectivity of transmissible spongiform encephalopathies from blood. *Lancet* 2004; **364**:529–531
- 14 Barz D: Detection of antigen structures in blood cells in differently prepared plasma presentations. *Anaesthesiol Reanim* 1994; **19**:155–158. German
- 15 Rutala WA, Weber DJ: Creutzfeldt–Jakob disease: recommendations for disinfection and sterilisation. *Clin Infect Dis* 2001; **32**:1348–1356
- 16 Foster PR: Removal of TSE agents from blood products. *Vox Sang* 2004; **87**:7–10
- 17 Hammond D, Lathrop J, Cervenakova L, Carbonell R (inventors): Prion protein ligands and methods of use. US patent WO 2004/050851A2; 3 December 2003
- 18 Biesert L, Suhartono H: Solvent/detergent treatment of human plasma – a very robust method for virus inactivation. Validated virus safety of Octaplas. *Vox Sang* 1998; **74**:207–212