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Pathogen safety and characterisation of a highly purified human alpha$_1$-proteinase inhibitor preparation

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ABSTRACT

Alpha$_1$-proteinase inhibitor (A_1PI) deficiency is a genetic condition predisposing to emphysema. Respreeza/Zemaira, a therapeutic preparation of A_1PI, is prepared from human plasma. This article describes the purity and stability of Respreeza/Zemaira and the capacity of virus and prion reduction steps incorporated into its manufacturing process.

Purity and stability of Respreeza/Zemaira were analysed using established methods. To test pathogen clearance capacity, high levels of test viruses/prions were spiked into aliquots of production intermediates and clearance studies were performed for selected manufacturing steps, under production and robustness conditions, using validated scale-down models.

Respreeza/Zemaira had a purity of 99% A_1PI and consisted of 96% monomers. It remained stable after storage for 3 years at 25 °C. Specific activity was 0.895 mg active A_1PI/mg protein. Pasteurisation inactivated enveloped viruses and the non-enveloped hepatitis A virus. 20 N/20 N virus filtration was highly effective and robust at removing all tested viruses, including parvoviruses, to below the limit of detection. Cold ethanol fractionation provided substantial reduction of prions.

The manufacturing process of Respreeza/Zemaira ensures the production of a stable and pure product. Taking into consideration the donor selection process, the testing of donations, and the highly effective virus and prion reduction, Respreeza/Zemaira has a high safety margin.

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1. Introduction

Alpha$_1$-proteinase inhibitor (A_1PI) deficiency is a genetic condition that, together with environmental factors, predisposes individuals to emphysema [1]. A_1PI is the principal antiprotease in the lower respiratory tract, where it inhibits neutrophil elastase, an enzyme that destroys pulmonary tissue.

A therapeutic preparation of A_1PI, manufactured by CSL Behring (Kankakee, IL, United States [US]), is approved for the indication of chronic augmentation and maintenance therapy in individuals with A_1PI deficiency and clinical evidence of emphysema under the trade name Zemaira in the US (2003), New Zealand (2011), Puerto Rico (2011), and Brazil (2012). In the European Union (2015), under the trade name of Respreeza, it is indicated for maintenance treatment, to slow the progression of emphysema in adults with documented severe A_1PI deficiency. Respreeza is also approved in Switzerland (2016) for the

Abbreviations: ADA, anti-drug antibody; A_1PI, alpha$_1$-proteinase inhibitor; B19V, parvovirus B19; BVDV, bovine viral diarrhea virus; CJD, Creutzfeldt-Jakob-Disease; CPV, canine parvovirus; DTT, dithiothreitol; EMA, European Medicines Agency; FDA, Food and Drug Administration; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus; HIC, hydrophobic interaction chromatography; HIV, human immunodeficiency virus; IEC, ion exchange chromatography; NAT, nucleic acid amplification technology; PPTA, Plasma Protein Therapeutics Association; PrP^{Sc}, purified proteinase-resistant pathogenic prion protein; PRV, pseudorabies virus; RP-HPLC, reversed-phase high-performance liquid chromatography; SE-HPLC, size-exclusion high-performance liquid chromatography; US, United States; vCJD, variant Creutzfeldt-Jakob-Disease; WHO, World Health Organization; WNV, West Nile virus.

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indication of maintenance treatment in adults with severe A_1PI deficiency and slowing the underlying destruction of lung tissue leading to emphysema.

The A_1PI in Respreeza/Zemaira is prepared from human plasma. All biologically derived therapeutics carry a low potential residual risk for the transmission of blood-borne viruses [2] and, theoretically, prions [3]. Therefore, the manufacturing process of Respreeza/Zemaira includes multiple complementary safety measures to ensure that the residual risk is minimised for both known and emerging viruses: 1) plasma donations are sourced from healthy, low-risk donors and tested by serology and nucleic acid amplification technology (NAT) for the absence of certain viruses (human immunodeficiency virus [HIV]-1/-2, hepatitis A virus [HAV], hepatitis B virus [HBV], hepatitis C virus [HCV], and parvovirus B19 [B19V]), 2) the plasma pool for fractionation is tested for the absence of certain viruses (see above), and 3) the manufacturing process includes validated effective manufacturing steps capable of inactivating/removing a broad spectrum of viruses [2,3,4].

In this article, we report the results of pathogen clearance studies that were conducted to test the efficacy of the pathogen clearance steps incorporated into the manufacturing process, using validated scale-down models of the commercial manufacturing steps. The pathogen clearance studies comprised virus validation studies and prion evaluation studies. We also present the bioanalytical characteristics of Respreeza/Zemaira and investigate the effect of long-term storage on the purity of the product. In addition, the potential formation of anti-drug antibodies (ADAs) in treated patients, a known risk for protein products, was assessed in samples from the randomised, controlled RAPID trials [1,5], using an immunogenicity assay (these data have not previously been published).

2. Material and methods

2.1. Bioanalytical characteristics

Reversed-phase high-performance liquid chromatography (RP-HPLC), size-exclusion high-performance liquid chromatography (SE-HPLC), and SDS-PAGE under reducing conditions were carried out as described previously [6], in order to assess the purity of Respreeza/Zemaira. For quantification of the SDS-PAGE results, A_1PI percentages were calculated by comparing the percentage area of the A_1PI band to the total peak area based on densitometry (Beckman Appraise). Western blot analysis was performed using polyclonal anti-A_1PI (human) and polyclonal anti-whole human serum. The functionality of A_1PI protein was determined by measuring the elastase inhibitor capacity of A_1PI using a chromogenic substrate, and comparing to an A_1PI standard calibrated against the World Health Organization (WHO) international standard [7]. The WHO standard derived value was then adjusted by dividing it by 1.089 to maintain consistency with internal results prior to the WHO standard release. Specific activity was calculated as the ratio of mg active A_1PI to mg protein, where protein concentration was determined by optical density using an extinction coefficient of 4.33 $(cm)^{-1}$ $(g/100\ mL)^{-1}$ [6]. Specific impurities (albumin, $alpha_1$-acid glycoprotein, $alpha_2$-macroglobulin, apolipoprotein, antithrombin III, ceruloplasmin, haptoglobin, IgA, IgG, transferrin) were evaluated by immunonephelometry (Siemens BN II nephelometer).

The stability of Respreeza/Zemaira after 3 years of storage at 25 °C was determined by analysing potency, and by SDS-PAGE and SE-HPLC.

2.2. Immunogenicity

The immunogenicity of A_1PI was assessed by testing for the presence of ADAs (see Supplemental Text File 1) in the serum of 177 subjects who received Respreeza/Zemaira during clinical trials (RAPID trials [1,5]). The procedures followed in the clinical trials were in accordance with the ethical standards of the Independent Ethics Committees/Institutional Review Boards, which included obtaining written informed consent.

2.3. Scale-down models used to determine pathogen clearance capacity

Pathogen clearance studies were conducted using validated scale-down models of the commercial manufacturing steps. The manufacturing process of Respreeza/Zemaira, upon which the scale-down models were based, is described in Supplemental Text File 2 and Supplemental Fig. 1. The scale-down experiments (range of 4000 to 37,000-fold scale-down) were carried out at volumes of 92 mL for cryoprecipitation, 176 mL for 8% and 159 mL for 20%-ethanol precipitation, and 63–65 mL for virus filtration.

Process parameters, such as temperature, pH, buffer composition, process time, filter loads, pressures, and rinse volumes (the latter three for the virus filtration step) were controlled tightly to meet manufacturing conditions.

For the virus filtration step, protein yields, protein concentration, potency yield, specific activity, filtration times, and percentage of monomers were compared to the production-scale process. For pasteurisation, the stabiliser excipients were adjusted prior to spiking, such that the final concentration of excipients after spiking matched the manufacturing conditions. The scale-down design was considered validated since the ranges of the potentially critical process parameters were met.

2.4. Virus validation studies using the scale-down models

The efficiency of virus reduction by the pasteurisation and virus filtration steps was investigated in virus validation studies performed according to regulatory guidance [2,4], meeting good laboratory practice requirements. The experiments were performed in specially designed virus laboratories remote from the manufacturing facilities. High levels of virus were spiked into aliquots of production intermediates, and then pasteurisation or virus filtration was performed using the validated scale-down model. The volume of the virus stock added to the aliquot never exceeded 10% (v/v) of the total sample volume. In accordance with regulatory guidelines, at least two independent virus validation runs were performed for each virus under the routine manufacturing conditions [8] using starting material from two different production lots.

The viruses that were assessed, shown in Table 1, were chosen because they could potentially be present in blood plasma or serve as a model for other blood-borne viruses in accordance with European Medicines Agency (EMA) guidelines [2,4]. High titre virus stocks were prepared, titrated, and prefiltered as described previously [3]. The infectivity titre was calculated according to the Spearman-Kärber method [11]; see Supplemental Text File 3 for details. An initial virus titre of $\geq$5.1 $\log_{10}$ $CCID_{50}$/mL for pasteurisation and virus load of $\geq$6.5 $\log_{10}$ $CCID_{50}$ for virus filtration was attained after spiking. Calculations of the virus filtration were based on the virus load instead of the virus titre to consider the change in volume.

To test the robustness of the pasteurisation step, critical process parameters were assessed beyond those specified for the manufacturing process:

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