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Research Article

Quality and Batch-to-Batch Consistency of Original and Biosimilar Epoetin Products

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ABSTRACT

Comprehensive physicochemical characterization and biological assays are essential parts in assessing quality attributes of biologicals. Here, we compared the quality of different marketed recombinant human erythropoietin (epoetin) products: originators, Eprex and NeoRecormon as well as 2 biosimilars, Retacrit and Binocrit. In addition, assessment of batch-to-batch variability was included by collecting 2 or more batches of each product. Common assays which included sodium dodecyl sulfate–polyacrylamide gel electrophoresis, high-performance size-exclusion chromatography, asymmetrical flow field–flow fractionation, capillary zone electrophoresis, and potency testing were used. Of the tested products and among batches of single products, variations in epoetin content, isoform profiles, and potency were found. Ultimately, this study demonstrated the high quality of epoetin products with some degree of variation among products and batches, confirming the "similar but not identical" paradigm of biologicals.

Introduction

Since the 1980s, the advent of recombinant DNA technology has enabled the development of many innovative recombinant human therapeutic proteins.[1,2] These products have enabled the treatment of a variety of diseases and have become the fastest growing class of therapeutics. Recombinant human erythropoietin (epoetin) was one of the first authorized recombinant proteins on the market. It is mainly used for the treatment of anemia in patients with chronic kidney disease and cancer.[3,4]

Severe side effects, such as thromboembolic processes and antibody-associated pure red cell aplasia (PRCA) are rare. PRCA may occur if epoetin-induced antibodies are able to neutralize the native endogenous erythropoietin.[5,6] Epoetin shares its factors for immunogenicity with nearly all therapeutic proteins. The exact mechanisms underlying immunogenicity are still not fully understood. Multiple factors including product-related factors (formulation, contaminants, glycosylation and impurities), storage and handling, route of administration, and patient characteristics play a role in this.[7,8]

Since 2006, the loss of patent and data protection has allowed the introduction of generic versions of therapeutic proteins such as somatropin, filgrastim, and epoetin. However, the generic regulatory route used for small molecules cannot be used for proteins. Owing to their inherent variability, complexity, and heterogeneity, it is impossible to establish that 2 protein products are identical.[9,10] Individual protein products themselves also demonstrate microheterogeneity and batch-to-batch variability so cannot be identical to themselves. Therefore, regulatory frameworks have been established throughout the world requiring an extensive comparison in quality, efficacy, and safety to show similarity between the original product and the intended copy.[11,12] If the criteria are met, the duplicate product can be marketed as a biosimilar.

As we had access to 4 marketed epoetin products, 2 originators, Eprex and NeoRecormon, and 2 biosimilars, Retacrit and Binocrit, we performed quality assessment for these products. Eprex (epoetin alfa) and NeoRecormon (epoetin beta) have been reported to differ in their isoform compositions and biological properties on

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account of the use of different CHO cells strain.[13] Meanwhile, the quality assessment of Retacrit and Binocrit to their reference product, Eprex, has been shown elsewhere to have slight variation in their quality attributes.[14,15]

Besides quality, batch consistency is also considered important for biologicals. Although a few studies have looked into batch-to-batch variability of an individual epoetin brand,[13,16,17] there has been no published study on batch-to-batch consistency of multiple epoetin brands marketed in Europe. As we also had the possibility to collect multiple batches from these 4 epoetin products, this comparability study is feasible as a follow-up to a study we published earlier.[14]

Materials and Methods

Epoetin Products

All epoetin products (see Table 1 for an overview) were either obtained from local pharmacies in the Netherlands or provided by Hospira and Sandoz. They were received in the original prefilled syringes and stored as stated on the product specification. As an internal reference standard, epoetin-biological reference preparation (BRP) batch 3 (EDQM, Strasbourg, France) was included in every experiment to validate the method as recommended in the European Pharmacopeia (Ph. Eur.) monograph on Erythropoietin concentrated solution.[18] It contains equals parts of epoetin alfa and beta.[19] Before every test, visual inspection was performed for the potential presence of visible particles. All products remained clear and colorless. In all cases, products were tested within their shelf lives.

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

The epoetin products were loaded on 5% polyacrylamide gel (stacking section) and separated on 15% polyacrylamide gel (running section) under nonreducing conditions as previously described by Brinks et al.[14] Unless indicated otherwise, all materials were obtained from Bio-Rad Laboratories B.V. (Veenendaal, the Netherlands). In short, loading solutions of all epoetin products included 24 μL of undiluted products and 6 μL of 5× sample buffer (containing Tris-HCl pH 6.8, glycerol, sodium dodecyl sulfate and bromophenol blue). Two micrograms of epoetin-BRP batch 3 were included on each gel.

Before loading, all samples were incubated either at 95°C, 70°C, or room temperature (±25°C) for 10 min to facilitate protein unfolding. PageRuler™ Prestained Protein Ladder, 10-180 kDa (Life Technologies, Bleiswijk, the Netherlands) was used as a reference for molecular weight in all cases. Separation was performed on Mini-PROTEAN® II Electrophoresis Cell with the following running conditions: 30 min at 70 V, followed by 60 min at 150 V. Protein bands were visualized by silver staining method as described by Brinks et al.[14]

High-Performance Size-Exclusion Chromatography

During the course of this study, the collection of multiple batches of each epoetin product was rather difficult. Hence, epoetin products were obtained at different time points. Retacrit and NeoRecormon were obtained back in 2010. Subsequently, Binocrit and Eprex were obtained in early and late 2014, respectively. As there was an urge to analyze unexpired products, high-performance size-exclusion chromatography (HP-SEC) was first performed on a Waters 2695 Separations Module connected to a Waters 2487 Dual λ Absorbance Detector (Waters Corporation, Milford, MA) for the first 2 products. The machine was then no longer available, and we had to switch to an Agilent 1200 HPLC system (Agilent Technologies, Palo Alto, CA) combined with a Wyatt Eclipse (Wyatt Technology Europe GmbH, Dernbach, Germany) to analyze the later products.

On both machines, a Tricorn™ high-performance Superdex 200 10/300 GL column (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) was installed. Auto sampler (Agilent) temperature was set at 4°C, and each time, 100 μL of undiluted product were injected. The eluent was 14.4 g/L $Na_2HPO_4.2H_2O$ (Sigma-Aldrich, Zwijndrecht, the Netherlands), 0.2 g/L KH_2PO_4, and 23.4 g/L NaCl (Merck, Darmstadt, Germany) at pH 7.4 and filtered through a 0.2-μm filter (Sartorius Stedim, Göttingen, Germany).

Separation took place at a flow rate of 0.5 mL/min for 60 min at 30°C. Absorbance was recorded at 280 nm and analyzed using either Empower 2 software version 6.20.00.00 or Astra software version 5.3.4.20. A DAWN® HELEOS™ 18-angle laser light scattering (MALLS) was part of the Agilent system, therefore allowing estimation of the average molecular weight of eluting compounds. Alternatively, proteins with different molecular weights, (1) lysozyme, (2) trypsin, (3) ovalbumin, (4) albumin, and (5) holotransferrin, were used on the Waters system as calibration standards for molecular weight estimation. All proteins were purchased from Sigma-Aldrich.

Subsequently, the protein content was determined from the UV signal at 280 nm using Beer-Lambert law. For all epoetins, a molar extinction coefficient of 22,600 M^{-1} cm^{-1} was used.[20]

Table 1
List of All Epoetin Products

Brand Name (INN)	Lot Number	Declared Potency	Excipients
Eprex (epoetin alfa)	DDS5L00 DGS4W00 DHS5T00 DIS3M00	4000 IU/0.4 mL	Sodium dihydrogen phosphate dihydrate, disodium phosphate dihydrate, sodium chloride, glycine, polysorbate 80
Binocrit (epoetin alfa)	450112 730412 341211	10,000 IU/1.0 mL 8000 IU/0.8 mL	Sodium dihydrogen phosphate dihydrate, disodium phosphate dihydrate, sodium chloride, glycine, polysorbate 80
Retacrit (epoetin zeta)	8K058L8 8M072C9 9F081G9 9M108N9	10,000 IU/1.0 mL	Disodium phosphate dihydrate, sodium dihydrogen phosphate dihydrate, sodium chloride, calcium chloride dihydrate, polysorbate 20, glycine, leucine, isoleucine, threonine, glutamic acid, phenylalanine
NeoRecormon (epoetin beta)	H0002H01 H0003H01	30,000 IU/0.6 mL	Urea, sodium chloride, polysorbate 20, sodium dihydrogen phosphate dihydrate, disodium phosphate dodecahydrate, calcium chloride dihydrate, glycine, l-leucine, l-isoleucine, l-threonine, l-glutamic acid, l-phenylalanine