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RESEARCH PAPER

How Bio-questionable are the Different Recombinant Human Erythropoietin Copy Products in Thailand?

Liem Andhyk Halim • Vera Brinks • Wim Jiskoot • Stefan Romeijn • Kearkiat Praditpornsilpa • Anunchai Assawamakin • Huub Schellekens

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ABSTRACT

Purpose The high prevalence of pure red cell aplasia in Thailand has been associated with the sharp increase in number of recombinant human erythropoietin (rhEPO) copy products, based on a classical generic regulatory pathway, which have entered the market. This study aims to assess the quality of rhEPO copy products being used in Thailand.

Methods Twelve rhEPO copy products were purchased from pharmacies in Thailand, shipped under controlled cold chain conditions to the Netherlands and characterized using (1) high performance size-exclusion chromatography, (2) asymmetrical flow field-flow fractionation, (3) sodium dodecyl sulfate polyacrylamide gel electrophoresis in combination with (4) Western blotting and additionally tested for (5) host cell protein impurities as well as (6) endotoxin contamination.

Results Some of the tested rhEPO copy products showed high aggregate levels and contained a substantial amount of protein fragments. Also, one of rhEPO copy products had a high endotoxin level, exceeding the FDA limit.

Conclusions Our observations show that some of the tested copy products on the Thai market differ significantly from the originator rhEPO product, Epogen®. This comparison study supports a link between the quality attributes of copy rhEPO products and their immunogenicity.

KEY WORDS biosimilar · immunogenicity · protein characterization · pure red cell aplasia · recombinant human erythropoietin

ABBREVIATIONS

AF4	asymmetrical flow field-flow fractionation
CHO	Chinese hamster ovary
EPO-BRP	erythropoietin—biological reference product
FDA	Food and Drug Administration
HMW	high molecular weight
HP-SEC	high performance size exclusion chromatography
HSA	human serum albumin
LAL	limulus amebocyte lysate
LMW	low molecular weight
PRCA	pure red cell aplasia
R-AUC	relative-area under the curve
RHEPO	recombinant human erythropoietin
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis

INTRODUCTION

Recombinant human erythropoietin (rhEPO) is used worldwide to treat anemia in patients with chronic kidney disease. Like all other therapeutic proteins, rhEPO may show immunogenicity. Although the occurrence of antidrug antibodies in patients is rare, if they are formed, they may cross-react with endogenous EPO and may lead to pure red cell aplasia (PRCA). Between

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L. A. Halim • V. Brinks • H. Schellekens (✉)
Department of Pharmaceutics
Utrecht Institute for Pharmaceutical Sciences (UIPS)
Utrecht University, Universiteitsweg 99
3584 CG Utrecht, The Netherlands
e-mail: h.schellekens@uu.nl

W. Jiskoot • S. Romeijn
Division of Drug Delivery Technology
Leiden Academic Centre for Drug Research, Leiden University
P.O. Box 9502, 2300 RA Leiden, The Netherlands

K. Praditpornsilpa
Division of Nephrology, Faculty of Medicine, Chulalongkorn University
1873 Pathumwan, Bangkok, Thailand 10330

A. Assawamakin
Department of Pharmacology, Faculty of Pharmacy, Mahidol University
447 Sri-Ayuthaya Road, Rajathevi, Bangkok, 10400, Thailand

1998 and 2002, such an outbreak of anti-rhEPO antibody-induced PRCA took place (1).

Although the exact mechanisms underlying antibody-induced PRCA remain unknown, the increased incidence of PRCA has apparently been contributed to by the replacement of human serum albumin (HSA) by polysorbate 80 and glycine as stabilizer, and by the use of uncoated stoppers in Eprex® (2–4). More recently, PRCA has been attributed to tungsten-induced denaturation and aggregation of rhEPO (5). Overall it appears that product quality is the main determinant of the immunogenicity of rhEPO.

In addition to the originator rhEPO products, rhEPO biosimilars strictly by criteria evaluation or as intended copy products are available (6,7). Because the European Medicines Agency and the Food and Drug Administration (FDA) recognize the complexity of these products and consider generic drug approval guidelines unsuitable for market authorization, quality is ensured with tailored biosimilar guidelines that guarantee only products able to match the quality, efficacy, and safety of the originator product get market approval (8,9).

However, in developing countries such as Thailand, where biosimilar guidelines are still under development, rhEPO copy products have entered the market under a generic drug paradigm. Dozens of rhEPO copy products are on the Thai market, registered as generic products (10). Additionally, an increased incidence of PRCA in the Thai population has been shown by several reports (10,11). This increase in PRCA in Thailand might be due to a different genetic background of the patient population (12), or alternatively could be due to different product quality attributes (6,7,13). The latter has not yet been studied.

We compared the quality of 12 different copy rhEPO alpha products containing HSA available on the Thai drug market to the originator rhEPO alpha product, Epogen®. We employed high performance size-exclusion chromatography (HP-SEC) and asymmetrical flow field-flow fractionation (AF4) to assess monomer and aggregate content, and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) in combination with Western blotting to detect fragments and covalent protein aggregates. Finally, we determined the level of host cell protein impurities in these products as well as endotoxin contamination.

MATERIALS AND METHODS

RhEPO Products

Twelve different copy rhEPO alpha products manufactured worldwide were collected from one specific region in Thailand. As listed in Table I, all available copy products were purchased from pharmacies in Bangkok, Thailand, and shipped via a certified courier to Utrecht University, the Netherlands. Products were temperature monitored during shipping to ensure cold chain. Upon arrival, products were stored at 2–8°C and handled according to the manufacturers' specifications. Each product was inspected visually at the lab bench before analysis to check for the presence (if any) of visible particulates, since the products should be clear, colorless solutions.

Table I Origin and Declared Potency of Copy rhEPO Alpha Products Used in the Current Study. One Batch per Product was Used

10,000 international units/syringe	Korea 1
4,000 international units/syringe	China 1, China 2, China 3, India 1, Korea 2, Korea 3, Korea 4, Latin America 1, Latin America 2, Latin America 3 and Latin America 4

All testing was performed before the expiry date. Per product, one batch was used to compare its quality to the originator rhEPO product, Epogen® (Amgen, Thousand Oaks, California, USA). As all the tested copy products do contain HSA, Epogen® was considered a more suitable control than Eprex®. Epogen® still contains HSA in its formulation; both contain a comparable rhEPO alpha drug substance developed by Amgen and the two brand names indicate only that they are marketed by two different companies.

Sodium Dodecyl Sulfate Polyacrylamide Gel (SDS-PAGE)

To visualize proteins (including fragments and covalent aggregates), SDS-PAGE was performed under non-reducing and reducing conditions. Per rhEPO product, an amount corresponding to 2 IU was mixed with 1x NuPAGE® lithium dodecyl sulfate sample buffer with or without NuPAGE® Reducing Agent containing 50 mM dithiothreitol to a final volume of 12 μL. All samples were subsequently heated at 70°C for 10 min and loaded (10 μL) on a NuPAGE® Novex® 4–12% Bis-Tris Gel. The PAGERuler prestained protein ladder (ThermoFisher Scientific, Waltham, Massachusetts, USA) was used as molecular weight standard. Separation was performed at a constant voltage of 70 V for 30 min followed by 150 V for 60 min. After separation, proteins were visualized with a SilverQuest™ Silver Staining Kit. Unless stated otherwise, all products used were obtained from Life Technologies (Carlsbad, California, USA).

Western Blotting

After SDS-PAGE (described above), samples were transferred to an iBlot® Transfer Stack (nitrocellulose membrane, 0.2 μm)

using the iBlot® 7-Minute Blotting System (Life Technologies, Carlsbad, California, USA) following the manufacturer's recommendations. The membrane was incubated in blocking buffer (5% non-fat dry milk [ELK, Campina Melkunie, the Netherlands] in 1xTBS-$T_{0.05}$ [50 mM Tris–HCl, 150 mM sodium chloride and 0.05% (w/v) Tween 20, pH 7.4]) for 60 min at 25°C with constant orbital shaking. Following washing with 1xTBS-$T_{0.05}$, rhEPO or HSA was detected with a primary antibody directed against human EPO (custom-made rabbit polyclonal anti-EPO, 10 µg/mL, Biogenes, Germany) or an antibody directed against HSA (rabbit monoclonal anti-HSA, 1:5000, Bioke) in 5% bovine serum albumin by overnight incubation at 4°C. The blot was washed four times for 5 min with 1xTBS-$T_{0.05}$ and then incubated with anti-rabbit (goat polyclonal, horse-radish peroxidase labeled, 1:100000, Jackson ImmunoResearch, West Grove, Pennsylvania) for 60 min at RT. Upon washing for four times for 5 min with 1xTBS-$T_{0.05}$, the blot was exposed to SuperSignal West Femto Maximum Sensitivity Substrate (1:2 in phosphate buffered saline, ThermoFisher Scientific, Waltham, Massachusetts, USA) for 10 s at room temperature. Bands were visualized with a Gel Doc Imaging system equipped with a XRS camera and Quantity One analysis software (Bio-Rad, Hercules, California, USA).

High Performance Size-Exclusion Chromatography (HP-SEC)

Prior to measurement, the rhEPO products were diluted to a concentration of 2,000 IU/mL using Milli-Q water. Subsequently, 100 IU per sample was loaded onto a Tricorn™ high performance column Superdex 200 10/300 GL (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) installed on a Waters 2695 Separations Module (Waters Corporation, Milford, Massachusetts, USA) at 30°C. Because Western blotting showed the presence of HSA in the rhEPO products, both Erythropoietin Biological Reference Preparation (EPO-BRP) batch 3 (the European Directorate for the Quality of Medicines and HealthCare, Strasbourg, France) and human serum albumin (HSA, Sigma-Aldrich, St. Louis, Missouri, USA) were included as controls. Five different concentrations, ranging from 2.5 to 0.03 mg/mL of HSA and rhEPO were taken. The running buffer was composed of 1.5 mM potassium phosphate, 8.1 mM sodium phosphate and 0.4 M sodium chloride at a pH 7.4 and filtered through a 0.2 µm filter (Sigma-Aldrich, St. Louis, Missouri, USA) prior to use. Detection took place with a Waters 2487 Dual λ Absorbance Detector (Waters Corporation, Milford, Massachusetts, USA) at 280 nm. A gel filtration calibration kit (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom) was used to acquire a calibration curve. Data was collected and analyzed using the Empower 2 software version 6.20.00.00.

Asymmetrical Flow Field-Flow Fractionation (AF4)

In contrast to HP-SEC, AF4 is a matrix-free technique that uses an asymmetric cross flow to analyze proteins based on molecular weight. Proteins were separated on an Agilent 1200 apparatus (Agilent Technologies, Palo Alto, California, USA) with a UV detector (280 nm) and combined with a multiangle laser light scattering (MALLS) detector DAWN® HELEOS™ (Wyatt Technology Europe GmbH, Dernbach, Germany). Prior to measurement, all rhEPO products were diluted to 2,000 IU/mL in MilliQ-water. Fifty µL of all rhEPO products were injected through a channel equipped with a 350 µm spacer of medium width and a regenerated cellulose membrane with a cutoff of 5 kDa. The detector flow and the focus flow were set to 1 mL/min and 1.5 mL/min, respectively. The elution settings used are summarized in Table II. The running buffer was composed of 8.1 mM $Na_2HPO_4.2H_2O$, 1.5 mM KH_2PO_4 and 0.4 M NaCl at pH 7.4 (Sigma-Aldrich, St. Louis, Missouri, USA) and filtered through a 0.2 µm-pore polycarbonate filter (GE Osmonics Inc., Minnetonka, Minnesota, USA) before use.

The root mean square (rms) diameter was calculated from the MALLS signal, using the Berry Fit option of the Astra software version 5.3.2.22 (Wyatt Technology Europe GmbH, Dernbach, Germany).

Host-Cell Protein (HCP) Analysis

To quantify traces of Chinese hamster ovary (CHO) proteins in the rhEPO products, a CHO HCP ELISA Kit (Cygnus Technology, Southport, North Carolina, USA) was used. In short, 50 µL of the rhEPO products were placed in duplicate onto a 96-well plate pre-coated with affinity purified capture goat polyclonal anti-CHO antibodies. Samples were incubated with HRP-labeled goat polyclonal anti-CHO antibodies for 2 h at room temperature with constant orbital shaking (180 rpm). The supernatant was discarded and the plate was washed four times with 1x washing buffer (Tris buffered saline). Upon washing, plates were developed with 3,3′,5,5′-the tetramethylbenzidine substrate for 30 min without shaking. Development was stopped by adding 0.5 N sulfuric acid. Optical density was measured at 450/650 nm using

Table II AF4 Elution Program Settings

	Start (min)	Duration (min)	Cross-flow (mL/min)
Elution	0	2	1.8
Focus	2	1	1.5
Focus + injection	3	2	1.5
Elution	5	10	1.8
Elution	15	10	0

SPECTROstar^{Nano} (BMG LABTECH GmbH, Ortenberg, Germany). Values were corrected for background and a standard curve was constructed using the supplied CHO HCP standard. Data was fitted to a 4-parameter logistic fit using GraphPad. Subsequently, HCP content in each rhEPO formulation was determined.

Limulus Amebocyte Lysate (LAL) Endotoxin Assay

Endotoxin levels of the rhEPO products, including control Epogen®, were assessed with a colorimetric Limulus amebocyte lysate (LAL) assay (Lonza, Basel, Switzerland). All measurements were performed by Lonza (Verviers, Belgium) and samples were temperature-controlled transported to Lonza using a certified courier. The assay procedure was performed as follows. First rhEPO samples were diluted ten times in LAL reagent water, followed by heat treatment at 75°C for 30 min to inactivate HSA. Per product, a volume of 100 µL of a 1:100 dilution was dispensed onto a microplate well in duplicate. To verify that the products themselves would not interfere with endotoxin measurements, an additional plate was prepared using the same dilutions (duplicate) of each rhEPO copy product, and 0.5 endotoxin unit per milliliter (EU/mL) of *Escherichia coli* (*E. coli*) endotoxin stock solution was used to spike into the samples. The plates (spiked and unspiked) were tapped gently on the side repeatedly to facilitate mixing followed by incubation at 37°C for 10 min. Then, 100 µL of lysate solution was added to each well and incubated for an additional 10 min. Absorbance was immediately read at 405–410 nm using a Lonza Kinetic QCL Reader BE25-315 with WinKQC software. Distilled water was used to adjust the photometer to zero absorbance. A standard curve with the best-fit straight line was generated using an *E. coli* endotoxin standard prepared at five different concentrations (50 EU/mL–0.005 EU/mL). The endotoxin concentration in each of the rhEPO samples was calculated based on the generated equation.

RESULTS AND DISCUSSION

Prior to characterization, all rhEPO products were examined visually. All products were clear and colorless solutions and contained no visible particulates.

SDS-PAGE

The molecular mass distribution of proteins was first investigated by SDS-PAGE. As shown in Fig. 1a, under non-reducing conditions, all products including controls displayed a band corresponding to monomeric rhEPO (molecular weight, 30.4 kDa) at an apparent size of 34–38 kDa This slight difference between expected molecular weight and the weight

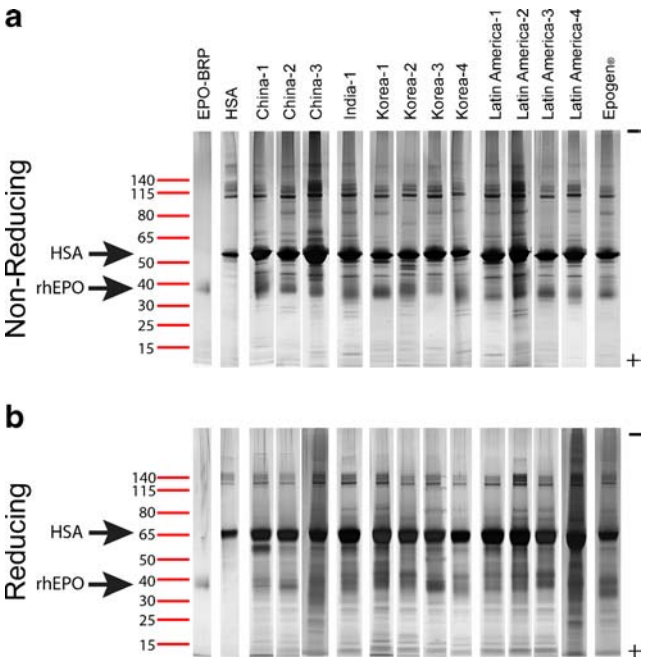

Fig. 1 SDS-PAGE gel of different rhEPO copy products (2 IU/lane) stained with SilverQuest™ Silver Staining under (**a**) non-reducing conditions and (**b**) reducing conditions. *Arrows* indicate the expected molecular weights of rhEPO or HSA.

indicated by the band on SDS-PAGE is consistent with previous reports (14–19). Principally, it is due to insufficient binding of SDS and lack of proper micelle formation (20). The thickness of the rhEPO monomer band was quite variable between products, suggesting difference in mass distribution and/or in the structure of glycoforms (21). In addition to the band corresponding to rhEPO monomer, all products showed another strong band at 50–60 kDa, which suggests the presence of HSA in their formulations. Above 80 kDa, a high molecular weight (HMW), additional bands/smears were present for all rhEPO copy products and for the originator rhEPO product, Epogen®. The pattern of this band/smear was similar to the HSA control indicating that the rhEPO products might contain HMW species due to HSA. However, it is also possible that rhEPO was present in these HMW species. Bands below 30 kDa, as observed for all rhEPO copy products and Epogen®, showed the presence of fragments and small oligomers within these products.

In comparison to Epogen®, most rhEPO copy products had comparable bands/smears. However, rhEPO copy products China 3 and Latin America 2 showed the most pronounced HMW bands above 80 kDa. Extra bands above 65 kDa were observed for Korea 2 and Latin America 3. Compared to Epogen® and the other rhEPO copy products, India 1, Korea 2 and Latin America 2 showed additional bands below 30 kDa.

Under reducing conditions, at ~40 kDa, all products also show a visible band for rhEPO monomer (Fig. 1b). At HMW

ranges, copy and control rhEPO products in general showed lighter tone smears compared to the smears found during non-reducing conditions, suggesting the breaking of HMW species. However, rhEPO copy products China 3 and Latin America 4 showed much darker tone smears, suggesting a higher amount of HMW fragments upon reduction. Inversely, all rhEPO copy products from Thailand and the control rhEPO showed darker tone smears below 65 kDa, suggesting the formation of fragments derived from either reduction of covalently linked aggregates or amino acid sequences that were cleaved off during manufacturing, handling and/or storage.

Western Blotting

As indicated by the SDS-PAGE results, like the originator product, Epogen®, in their formulations, all rhEPO products appeared to contain HSA. The presence of HSA as well as EPO monomer, potential aggregates and fragments in the products were further assessed by Western blotting. Under non-reducing conditions, monomeric rhEPO was detected around 40 kDa (Fig. 2a), as previously observed for SDS-PAGE (Fig. 1a). Additional bands just above 80 kDa were visible for all rhEPO copy products, in particular for Latin America 2. These bands suggest the formation of dimeric EPO-HSA, as confirmed by western blotting detected with anti-HSA antibody (Fig. 3a). in addition, rhEPO specific

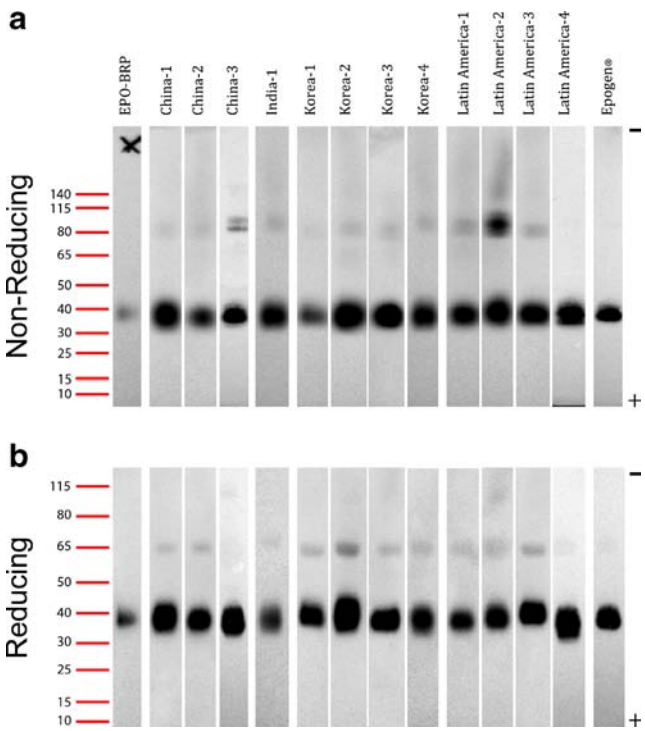

Fig. 2 Performance of various rhEPO copy products on SDS-PAGE (2 IU/lane) detected with Western blot using anti-EPO antibody under (**a**) non-reducing and (**b**) reducing condition.

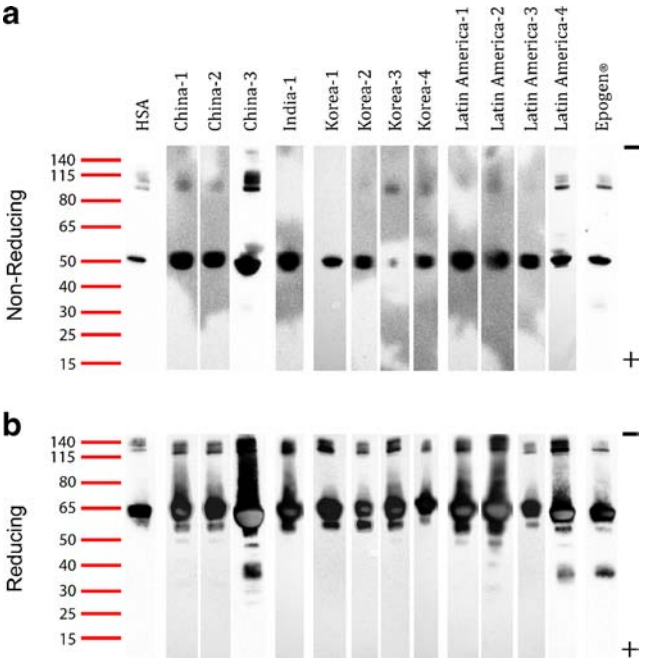

Fig. 3 Performance of various rhEPO copy products on SDS-PAGE (2 IU/lane) detected with Western blot using anti-HSA antibody under (**a**) non-reducing and (**b**) reducing condition.

smears at HMW ranges were also visible for rhEPO copy product Latin America 2, indicative of rhEPO-containing aggregates.

In the presence of a reducing agent (Fig. 2b), most formulations showed two bands at ~40 kDa and 65 kDa, indicating the presence of monomeric and dimeric rhEPO, respectively. Only China 3 displayed no band at ~65 kDa. Smears at higher molecular weight range and the 80 kDa band were no longer present, suggesting the reduction of covalently linked aggregates and dimeric rhEPO-HSA observed during non-reducing conditions, respectively.

When using a specific anti-HSA antibody, bands at 50 kDa and 65 kDa were present for both non-reducing and reducing conditions (Fig. 3a and b). This confirmed that, like Epogen®, all tested rhEPO products contained HSA. All products, including Epogen®, contained one or more additional bands with a molecular weight higher than 80 kDa, indicating the presence of aggregates containing HSA. Under non-reducing condition, the rhEPO copy product China 3 seemed to have the darkest tone bands in HMW region, indicating a higher concentration of HMW species as shown in Fig. 3a. Besides HMW species, the rhEPO copy products China 3 as well as Latin America 2, 4 and Epogen® showed additional bands below the monomeric HSA band under reducing conditions (Fig. 3b), suggesting fragments as a result from either reduction of covalently linked aggregates or amino acids that were cleaved off during manufacturing, handling and storage. No band was observed in the EPO-BRP lane, indicating the specificity of the antibody (data not shown).

HP-SEC

All rhEPO formulations were assessed for protein composition using HP-SEC. As Fig. 4 shows, both rhEPO and HSA monomer co-eluted at a similar retention time. Because the rhEPO and HSA peaks were indiscriminate, we focused only on fragments and HMW species identified per product. The first peak, eluting at 16 min, indicates HMW species is absent in the EPO-BRP and HSA sample. HSA trimer, dimer and monomer were detected at 23, 25 and 29 min (Fig. 4a). In the HSA-free formulation i.e., EPO-BRP, monomeric rhEPO was detected at 30 min. Meanwhile, a peak eluting at 29 min corresponded to the co-elution of between HSA and rhEPO monomers in all rhEPO copy products and Epogen®. Additional peaks eluting at 16 min and 24 min were possible rhEPO/HSA HMW and dimer, respectively. The other two peaks eluted at 39 min and 49 min were considered as low molecular weight species. They are likely formed due to formulation excipients and/or short amino acid sequences that might have cleaved off the rhEPO molecule. The chromatograms of all rhEPO copy products resemble Epogen®, except the rhEPO copy product Latin America 3, which differed from other products since a peak was visible at 45 min instead of 49 min.

Figure 5 summarizes the areas under the curve (R-AUC) of these peaks relative to the total AUC for each product. Overall, most of the rhEPO copy products, compared to Epogen®, have a lower or similar percentage of HMW species. RhEPO copy product Korea 2 seems to contain the least amount of HMW species of all tested rhEPO products, while rhEPO copy product China 3 has the highest percentage (~4%). In addition, the R-AUC of low molecular weight species was also quantified in Figure S1.

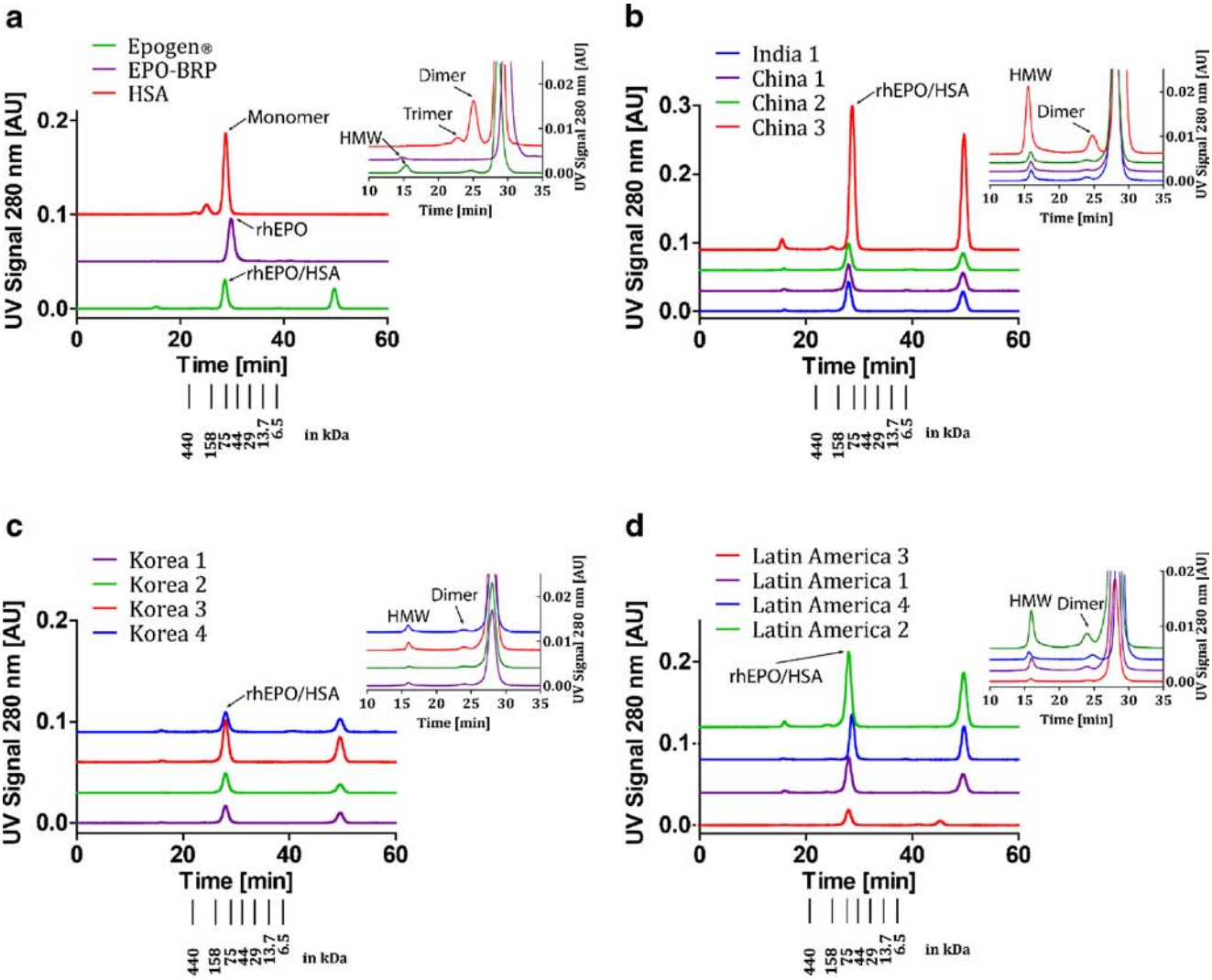

Fig. 4 HP-SEC chromatograms of (**a**) HSA, EPO-BRP and Epogen®, and different rhEPO copy products from (**b**) China and India, (**c**) Korea and (**d**) Latin America, using UV detection at 280 nm. In all copy products and Epogen®, the dimer and HMW are likely to contain both rhEPO and/or HSA. The loaded amounts of EPO-BRP and HSA were 50 μg (~5706 IU) and 125 μg, respectively.

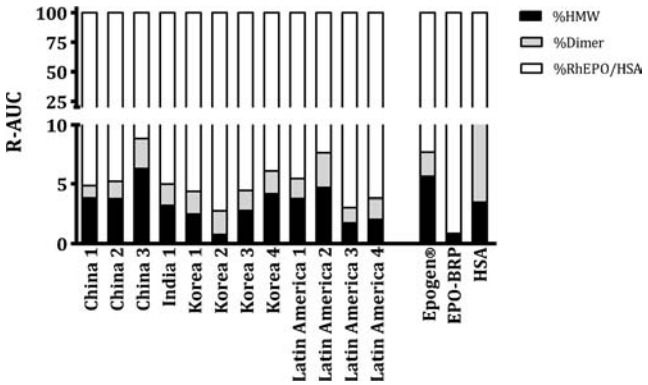

Fig. 5 *Bars* represent the areas under the curve (R-AUC) of the HMW, dimer and rhEPO/HSA peaks relative to the total AUC for each product.

AF4

AF4 was used as an orthogonal method to HP-SEC to assess the quality of the rhEPO products. Unfortunately, this analysis also showed the peaks corresponding to EPO monomer and HSA monomer as overlapping. Subsequently we focused on the presence of fragments and HMW species. Figure 6 shows HMW species detected after the cross-flow was stopped at 15 min, and such protein species were present in all rhEPO copy products and Epogen®. Similar to HP-SEC, HMW species may contain both rhEPO and HSA. Interestingly, in control rhEPO, Epogen®, a distinct dimer peak was present in the co-elution peak of rhEPO and HSA monomer corresponding to possible HSA dimer. This peak was either (partly) present or absent for the other rhEPO copy products. It might be due to differences in protein quantity and/or different dimers of rhEPO as well as HSA. It also seems that the higher the HSA content the more overlap of the monomer tail and dimer tail.

While rhEPO copy products China 3, Korea 4 and Latin America 4 stand out with a high percentage of HMW species (~6%), other copy and control rhEPO products contain 5% or less of HMW species (Fig. 7). The estimated molecular weight of these HMW species, MALLS analysis revealed, was between 0.8 and 4×10^3 kDa. The molecular weight of rhEPO/HSA as well as distinct dimer peaks were also estimated, as shown in Figure S2. These data suggest that there are three rhEPO copy products with higher aggregate contents than other copy products and Epogen®, which might be a risk factor for immunogenicity (22). When compared to HP-SEC data, this AF4 data seems to give a higher estimate of HMW species

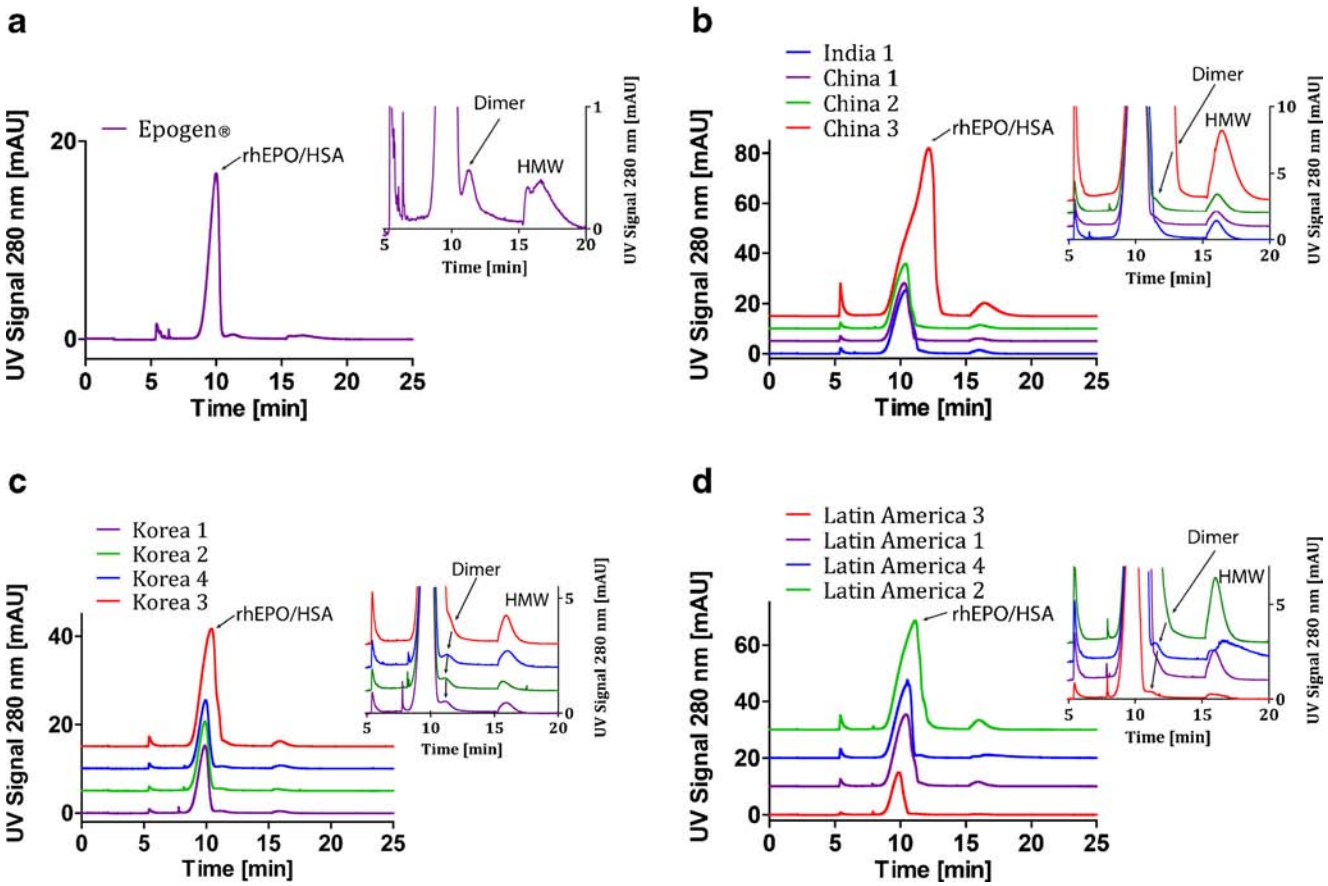

Fig. 6 AF4 elugrams of (**a**) Epogen® and different rhEPO copy products from (**b**) China and India, (**c**) Korea and (**d**) Latin America using UV detection at 280 nm. In all rhEPO copy products and Epogen®, the dimer and HMW are likely to contain both rhEPO and HSA.

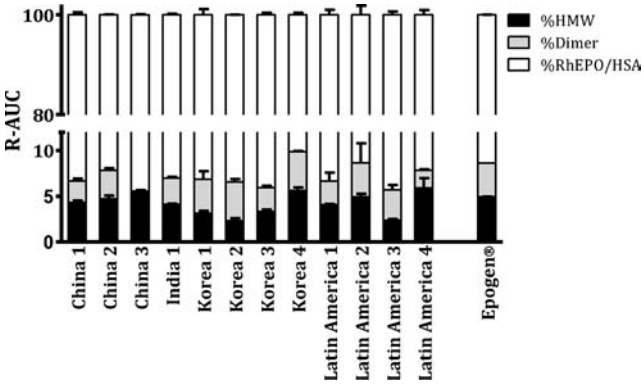

Fig. 7 *Bars* represent the areas under the curve (R-AUC) of the HMW, distinct dimer and rhEPO/HSA peaks relative to the total AUC for each product.

content (Figure S3). This, as Carpenter and colleagues have shown, may be because HP-SEC can underestimate the presence of HMW species too large to enter the matrix of the HP-SEC column (23). Moreover, better recoveries of HMW species in AF4 than in HP-SEC have been previously observed (24,25).

HCP

Host cell impurities may compromise therapeutic efficacy of therapeutic proteins, including rhEPO, and may result in adverse effects such as immunogenicity (26). As Fig. 8 shows, the amounts of CHO-derived proteins in the rhEPO products differ considerably. The product China 1 showed the highest level of HCP impurities while still remaining below the currently accepted limit of 100 ppm. Nevertheless, since a process or product specific HCP assays using anti-HCP antibodies specifically generated against the cell line or the product coming from a specific process was not available, the HCP values are most probably underestimated due to insufficient detection of all HCPs. Thus, elevated HCP values above the currently accepted limit of 100 ppm would be more meaningful than very low values as observed in this study.

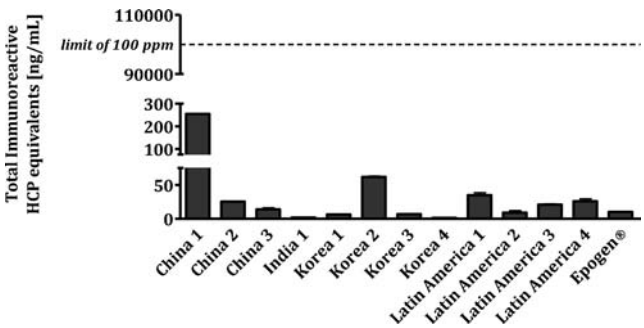

Fig. 8 The quantification of host cell protein contained in different copy and control rhEPO products.

Endotoxin Determination

Detecting endotoxin levels in (rhEPO copy) products is critical, as contaminants might be introduced during manufacturing, handling or storage. The presence of endotoxin in its drug formulation can result in pyrogenic responses and may affect immunogenicity of the finished products. For this reason, the endotoxin level was quantified in all rhEPO copy products and Epogen®. In most copy and control rhEPO products, endotoxin levels were below the detection limit of the assay. However, Latin America 4 had endotoxin levels exceeding the FDA limit (max. 0.5 EU/mL). This finding supports a previous publication by a group in Brazil in which 3 out of 12 rhEPO copy products from five different undisclosed suppliers showed substantially above FDA limits (27).

Gaps in Product Quality and Potential Safety

We have tested the quality of 12 different rhEPO products available on the Thai drug market. Although these products are used as more affordable and alternative treatment for renal anemia, their physicochemical properties show high aggregate rhEPO/HSA levels and contain a substantial amount of protein fragments for some of the rhEPO copy products. This could be a source of the higher risk of aggregate-induced immune response previously identified for these products (28,29).

Besides possible aggregate-induced immunogenicity, bacterial endotoxin can induce the production of anti-rhEPO antibodies leading to, in some cases, PRCA (30). In this study, we show that 1 out of 12 rhEPO copy products had high endotoxin levels, exceeding the FDA limit. Whether the differences mentioned above are correlated to the increasing PRCA incidence in Thailand remains speculative (10,11).

The international consensus is that the generic pathway, as adopted by the Thai regulatory agencies, is not suitable for these copy products. Instead, the biosimilar pathway pioneered by the EU and adopted by the WHO would be beneficial, as both biological and clinical data of copy products should be present and should exhibit similar quality, safety and efficacy to the originator rhEPO product. At present, these copy products should not be considered as biosimilars.

CONCLUSION

To our knowledge, this is the first comparison study of different rhEPO copy products gathered from one specific region, Thailand. Batch-to-batch variability in addition to potency testing and glycosylation analysis will be included in future studies as part of the quality assessment of the rhEPO copy products. Our data is the first and part of a collaborative prospective study trying to understand the link between

immunogenicity in Thai patients and product-related factors in rhEPO copy products on the Thai drug market. In combination with results on antidrug antibody incidences induced by these products (ongoing clinical study in Thailand), a link can hopefully be established. Until then, these rhEPO copy products remain bioquestionable.

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