Efficacy of recombinant erythropoietins: is there unity of international units?

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The European Medicines Evaluation Agency (EMEA) has developed guidelines to ensure similarity, ‘in terms of quality, safety and efficacy’, between similar biological medicinal products (biosimilars) in comparison to the originators (http://www.emea.eu.int). The quality and safety of biosimilar recombinant human erythropoietins (rhEPOs) has been previously discussed [1–3]. The present comment focuses on the comparability of the in vivo potency of rhEPOs, which is relevant to the economic use of erythropoiesis stimulating agents (ESAs), because biosimilar rhEPOs have been priced by up to 30% lower than the originators in some countries of the European Union (EU).

Therapeutic rhEPOs

According to the International Nonproprietary Name (INN), experts of the World Health Organization (WHO), ‘epoetins’ are rhEPOs with the same amino acid sequence and glycosylation sites as endogenous EPO [4]. Amino acid changes are indicated by distinct prefixes (e.g. ‘darbepoetin’) and glycan differences by Greek letters. The composition of the glycans of rhEPOs depends on the transfected cell culture and product purification procedures [5]. Indeed, the originator epoetins alfa and beta, which are engineered in Chinese hamster ovary (CHO) cells, exhibit differences in the sugar composition of their N- and O-glycans [6,7]. However, one epoetin alfa biosimilar (HX575) has received EMEA approval as ‘epoetin alfa’, although it contains more high-mannose structures and less N-glycolyl-neuraminic and diacetylated neuraminic acids than the originator (Eprex®/Erypo®) [8]. A second epoetin alfa biosimilar (SB309), which has received the INN ‘epoetin zeta’, has less O-glycans, N-glycolyl-neuraminic acid and O-acetyl neuraminic acid than the reference product [9].

Definition of the EPO unit

EPO amounts are expressed in units (U) rather than in grams or moles, because native EPO and rhEPOs are mixtures of isoforms with differing bioactivities [6,7]. As per definition, one EPO unit elicits the same erythropoiesis-stimulating response in rodents (historically: fasted rats) as five micromoles of cobaltous chloride. The ‘international unit’ (IU) for EPO was introduced, when EPO Standard B (from human urine; replaced Standard A from sheep plasma) was established as the First International Reference Preparation (IRP) [10]. In 1972, the second IRP of human urinary EPO (specific activity: 2 IU/mg glycoprotein) was implemented by the National Institute for Biological Standards and Control (NIBSC), UK [11]. In 1992, the NIBSC established a standard for purified rDNA-derived human EPO (87/684; 130 000 IU/mg glycoprotein) on behalf of the WHO [12]. The NIBSC report notes major differences in reactivities between in vivo, in vitro biological and immuno-logical assays with the various urinary and rhEPOs tested [12]. Hence, the second IRP was recommended for assaying native human EPO, and the rDNA-derived standard for assaying rhEPOs [12]. EPO levels should be expressed in units (U) but not in international units (IU), unless the results are obtained by the in vivo bioassay utilizing an EPO standard previously calibrated in vivo against one of the international EPO standards [13].

The European Directorate for the Quality of Medicines has established separate rhEPO biological reference preparations (BRPs; presently batch 3) for therapeutic rhEPOs [14]. BRPs are 50:50 blendings of originator epoetin alfa and epoetin beta. Because comparative measurements against BRP2 and the second IRP were not consistent, the potency of BRP3 was assigned against BRP2 to guarantee continuity of unitage between the successive BRP batches [15].

In vivo EPO bioassays

The in vivo bioassays for EPO are commonly performed in polycythaemic or normocytic mice [13]. In polycythaemic mice, endogenous EPO production is suppressed by previous red blood cell transfusion or exposure to hypoxia (normo- or hypobaric). The assay in normocytic mice...
mice suffices for routine pharmaceutical quality control of rhEPO, although its detection limit is relatively high. The in vivo bioassays require many animals, are time-consuming and expensive, and there is a considerable intra- and interassay variation of the results [16]. For example, the estimates of the second IRP from 25 independent assays in 12 different laboratories ranged from 46 to 132 IU (geometric mean: 86 IU) EPO per ampoule [12].

According to the European Pharmacopoeia (Ph Eur monograph 1316), the activity of therapeutic rhEPOs is compared to the BRP by either method A (measurement of incorporation of $^{59}$Fe into red blood cells 4 days after EPO injection in mice made polycythaemic by exposure to hypobaric hypoxia) or method B (microfluorometrical measurement of reticulocytes in a flow cytometer 4 days after EPO injection in normocytocenic mice) [17]. Owing to the poor accuracy of the assays, the Ph. Eur monograph notes: 'The estimated potency is not less than 80% and not more than 125% of the stated potency. The fiducial limits of error of the estimated potency are not less than 64% and not more than 156% of the stated potency.' [17]. This wide range implicates that there can be major inter-batch differences in the activities of epoetins of the same brand and – even more so – of the products from different manufacturers. For example, an ampoule labelled 2000 IU may contain 1600 or 2500 IU rhEPO. Depending on the location and date of the production of therapeutic rhEPOs, different standards (second IRP, BRP2, BRP3) have been applied by pharmaceutical companies. There is another risk of bias. The manufacturers are forced to use in-house standards for the calibration of new therapeutic lots, because the international reference preparations are limited and not intended for routine use in bioassays.

None of the conventional bioassays is suitable for valid quantification of the activities of the second-generation ESAs, darbepoetin alfa (Aranesp®) and methoxy polyethylene glycol–epoetin beta (methoxy-PEG epoetin beta; Mircera®) [18]. The terminal half-life of i.v. administered darbepoetin alfa is 3- to 4-fold longer than that of the epoetins (25 versus 6–9 h) [19,20]. The methoxy-PEG epoetin beta has an even longer half-life (130–140 h) [21]. Since the in vivo bioassays are inappropriate for detection of such long-acting ESAs, darbepoetin alfa and methoxy-PEG epoetin beta are dosed in micrograms instead of IU. As the specific activity of rhEPO amounts to about 200 000 IU/mg peptide, 1 µg of darbepoetin alfa or methoxy-PEG epoetin beta peptide corresponds biophysically to 200 IU rhEPO peptide. It is beyond the present comment to consider the critical issue of reduced dose requirements for the second-generation ESAs, although they allow for less frequent application.

### Potency of follow-on epoetins

HX575 (INN: epoetin zeta; Retacrit®, Silaplo®) is another biosimilar to Eprex®/Erypo® [9]. When tested in normocytocenic mice, the relative potencies of two different batches of SB309 and Eprex®/Erypo® were 0.86 and 1.02, respectively. The relative potency of BRP2 versus Eprex®/Erypo® was 1.04. The therapeutic equivalence of SB309 and Erypo® was investigated in two studies in CRF patients [9]. In the correction study, the mean haemoglobin values of the last 4 weeks were 11.6 g/dl with either drug. However, the mean weekly dosage of SB309 needed was ~10% higher than that of Erypo® (182 versus 166 IU/kg/week). In the maintenance study, following a switch from Erypo® to SB309 the dose increased by 10–15% and the haemoglobin level decreased transiently by 5%. After a switch from SB309 to Erypo®, the dose decreased by 10% and the haemoglobin level increased simultaneously by 10% [9]. The Erypo® batches contained on average 9% and the SB309 batches 1% over the labelled amount of protein. The specific activities for both products were similar (131 IU/µg). Thus, the observed difference in efficacy can be related to a lower syringe content (in terms of bioactivity and the protein) of the SB309 versus the Erypo® test batches despite the same nominal dose, resulting from the different bioassays for determination of EPO activity for the test product (normocytocenic mouse as a bioassay preferably used in the EU) and the reference product Erypo® (exhypoxy polycythaemic mouse bioassay used in the USA). Whether a comparison of new batches of the two drugs would yield the same difference in potency remains an open question.

Epoetin alpha copies are available from many manufacturers in Asia, Africa, non-US America and non-EU Europe [22]. In an investigation of 12 copies of epoetin alpha from five different manufacturers, potency values ranging from 68 to 119% were assessed by the bioassay in normocytocenic mice [23]. In a study of 11 copies of epoetin alpha from eight manufacturers outside of the EU and the USA, activities higher than specification (137–226%) were determined in four samples and activities lower than specification (71–75%) in two samples by an exhypoxy polycythaemic mouse assay [24]. In addition, there were major batch-to-batch differences in the potency of rhEPOs from the same manufacturers [24].

### Conclusions

rhEPOs have been used for 20 years for prevention and reversal of anaemia in CRF, malignancy, AIDS and surgical interventions. Copied rhEPOs have been available for years in countries where the patent rights are not applicable. Biosimilar rhEPOs have been approved in the EU. One selling point of a biosimilar epoetin is its price. However, there
is a second economic criterion, namely the erythropoietic potency. All of the products are calibrated in mice by the bioassays that are not precise enough to ensure identical clinical efficacy. In vitro, the assays provide even less information in this regard. rhEPO potencies have been queried with respect to follow-on epoetins because these are compared to an originator product in the marketing approval process. Basically, however, the problem of precise calibration of rhEPOs holds likewise true for the originator products.

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References


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