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Assessing the bioequivalence of biosimilars

The Retacrit® case

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This first phase of the first generation of modern biotechnology-derived protein drugs is now coming to an end with the expiration of their patents and consequently the possibility of the marketing of copies. The generic paradigm used for classical drugs cannot, however, be applied to therapeutic proteins because of their complexity. The European regulatory system issued its first comprehensive guidelines for the development of the so-called biosimilars. A number of these products have now been introduced to the European market. The case of Retacrit®, one of the biosimilar epoetines, is being discussed as an example of the challenges encountered when assessing the bioequivalence of therapeutic proteins.

Introduction

In 1982, human insulin was introduced as the first recombinant-derived therapeutic protein. Since then more than 200 different protein drugs have been introduced and this class of therapeutics has become firmly established. Many of these drugs are life-saving and/or greatly improve the quality of life. During these 26 years, the production and purification methods of these complicated and unstable molecules have been greatly improved and also clinical experience has accumulated.

This first phase of the first generation of modern biotechnology-derived protein drugs is now coming to an end with the expiration of their patents and consequently the possibility of the marketing of copies. With classical drugs, marketing authorization can be achieved by showing that the generic is chemically identical to the innovator drug and is bioequivalent by showing the pharmacokinetics to be comparable.

The generic paradigm cannot, however, be applied to therapeutic proteins in an analogous manner [1]. Protein pharmaceuticals are mostly large complex molecules and current analytical methods do not allow their full physical characterization. Moreover, even the most sophisticated analytical tools are not sensitive enough to predict fully the biological and clinical characteristics of the product. In general, they also show heterogeneity due to natural processes in the host cells needed for their production, such as variations in glycosylation or protein clipping. Also, modifications can be introduced during production, purification, formulation and storage. Besides these product-related impurities, host cells and (biological) materials used during production and purification, such as resins and monoclonal antibodies for affinity chromatography, may introduce process-related impurities.

All these impurities and contaminants may influence the biological and clinical properties of the final product. Therefore, all different steps of production and purification need to be extensively monitored. In addition, this process is dynamic: for many products and especially the first group for which the patents will expire shortly, it is expected that the production process has undergone continuous improvement so the current product may differ substantially from the initially introduced product.

The clinical and biological properties of a protein drug are the result of its basic properties such as amino acid sequence and three-dimensional structure, as well as the specific production, purification, formulation and storage conditions. Regulatory authorities require manufacturers to show that they control the production process and are capable of reproducibly manufacturing batches that meet the product specifications established through detailed characterization.

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Modifications of the established process, which occur during the life cycle of the product, are only accepted if the manufacturer shows that the product of the new process is comparable to the initially manufactured product [2]. Comparability studies include characterization of the old and new product in side-by-side analyses by state-of-the-art methods, stability studies and, if needed, may include preclinical and clinical studies examining the pharmacokinetic, pharmacodynamic and immunogenic properties of the new product and even efficacy and safety studies. The goal of these comparability studies is not to show that the quality attributes of the prechange and postchange products are identical, but that they are highly similar.

So a company planning to market a copy after the expiration of the patent of an original product faces a number of problems. Few of the specifics of the production process and the analytical methods used are available in the public domain and are proprietary knowledge. The competitor also has no access to the in-house standards and the preformulation material necessary to compare.

Because the generic approach is not applicable to protein drugs, the term, similar biological medicinal products, has been introduced as the official terminology in the EU while the FDA uses the term, follow-on biological products. But the term biosimilars has become the preferred terminology both in scientific and regulatory discussions and publications.

European guidelines and regulations

The only regulatory agency that has issued guidelines up to now has been the European Medicine Evaluation Agency (EMEA) based on the EU legislation, which became effective in November 2005. In this, biosimilars have been set apart from generic drugs [3]. Clinical data have become mandatory for a marketing authorization submission for biosimilars to the EMEA, which, like innovative biotechnology products, will need to be evaluated through a centralized procedure and not by national regulatory approval. The CHMP, the scientific committee of the EMEA, has issued a number of guidelines concerning the data required for marketing authorization.

EMEA/CHMP issued an overarching biosimilars guideline to set the scene and requirements for the development of biosimilars covering quality issues and non-clinical and clinical issues [4–6]. In addition, the EMEA/CHMP released four product-class specific guidelines [7–10]. The general overarching guideline introduces the concept of similar biological products, outlines the basic principles to be applied and provides applicants with a 'user guide' showing where to find relevant scientific information in the various EMEA/CHMP guidelines in order to submit a marketing authorization for a biosimilar.

The aim of the guideline on the quality aspects of similar biological medicinal products is to lay down the quality requirements for a biological medicinal product claiming to be similar to another one already marketed. Importantly, this guideline addresses the requirements regarding manufacturing processes, analytical methods to assess comparability, factors to consider when choosing a reference product and physicochemical and biological characterization of the similar biological medicinal product.

A biosimilar marketing authorization submission needs to contain the same extensive data on quality and safety as an innovative protein drug. In addition, the submission needs a supplement showing similarity in quality, safety and efficacy between biosimilar and the same reference product.

Comparison can be made against the official data, for example, pharmacopoeial monographs or against other published scientific data. Such comparisons at the level of both active substance and finished product are, however, limited and not sufficient to establish all aspects pertinent to the evaluation. Consequently, an extensive comparability exercise will be required to demonstrate that the similar biological medicinal product has a similar profile in terms of quality, safety and efficacy to the reference medicinal product. The generation of comparative data concerning physicochemical characteristics and preclinical testing will be complicated because unformulated bulk material of the innovator protein is unavailable to the biosimilar manufacturer. So the competitor can only use formulated marketed material. For some analytical techniques, isolation of the protein from the formulation is necessary. This may induce modifications, which can hamper the comparisons. Comparing with the biosimilar isolated from the same formulation is being used to compensate for these possible modifications.

The comparability exercise should include an assessment of the biological properties of the similar biological medicinal product and the reference medicinal product. The results of relevant biological assay(s) should be provided and expressed in units of activity calibrated against an international or national reference standard, when available and appropriate. These assays should comply with appropriate European Pharmacopoeia requirements for biological assays, if applicable.

The guideline on non-clinical and clinical issues of similar biological medicinal products addresses the pharmaco-toxicological assessment, the requirements for pharmacokinetic, pharmacodynamic, efficacy and safety studies, with emphasis on evaluation of immunogenicity of the similar biological medicinal product.

In addition, it requests an appropriate comparability exercise to demonstrate that the similar biological and reference medicinal products have similar profiles in terms of safety and efficacy.

Non-clinical and clinical studies

Before initiating clinical development, non-clinical studies should be performed. These studies should be comparative in nature and should be designed to detect differences in response between the similar biological product and the reference medicinal product and not just the response *per se*. Comparative pharmacokinetics studies designed to demonstrate clinical comparability between the similar biological medicinal product and the reference medicinal product with regard to key pharmacokinetic parameters are an essential part of the comparability exercise.

The design of comparative pharmacokinetic studies should not necessarily mimic that of the standard 'clinical comparability' design, since similarity in terms of absorption/bioavailability is not the only parameter of interest. In fact, differences in elimination characteristics between products, for example, clearance and elimination half-life should be explored.

The choice of the design for single dose studies, steady-state studies, or repeated determination of pharmacokinetic parameters should be justified by the applicant. Ordinary crossover design is

not appropriate for therapeutic proteins with a long half-life, for example, therapeutic antibodies and pegylated proteins, or for proteins for which formation of anti-drug antibodies is likely. The acceptance range to conclude clinical comparability with respect to any pharmacokinetic parameter should be based on clinical judgment, taking into consideration all available efficacy and safety information on the reference and test products. Hence, the criteria used in standard clinical comparability studies, initially developed for chemically derived, orally administered products may not be appropriate and the clinical comparability limits should be defined and justified before conducting the study.

Pharmacodynamic markers should be selected on the basis of their relevance in demonstrating the therapeutic efficacy of the product. The pharmacodynamic effect of the test and reference medicinal products should be compared in a population where the possible differences can best be observed. The design and duration of the studies must be justified. Combined pharmacokinetic/pharmacodynamic studies may provide useful information on the relationship between exposure and effect. The selected dose should be in the steep part of the dose-response curve. Studies at more than one dose level may be useful.

The latest general guideline to be issued by the EMEA/CHMP concerns the immunogenicity assessment of biotechnology-derived therapeutic proteins [11]. This guideline provides a broad overview of the immunogenic issues that biopharmaceutical companies must adequately address for the approval of a biosimilar product or when a manufacturing change occurs. The guideline discusses: factors that might influence immunogenicity and the potential consequences of immunogenicity; the development, design and interpretation of non-clinical and clinical assays to evaluate the immunogenic potency of a product, its comparability to other products and the implementation of a risk management plan. Many of the concepts discussed in the guideline will probably need to be adapted on a case-by-case basis.

Four product class-specific guidelines were issued for the development of biosimilars containing recombinant epoetin, somatotropin, human insulin and human granulocyte colony-stimulating factor. These documents outline preclinical and clinical data requirements for marketing approval, describing the size/design of the trials required and the best indication for demonstrating equivalence for each product, in comparison with a reference product.

One example of the product-specific guidelines is the EMEA/CHMP Annex on similar medicinal products containing recombinant erythropoietins. Interestingly, the regulatory requirements are more stringent for epoetins than for the other recombinant proteins, reflecting its greater molecular complexity and clinical history, especially the antibody caused pure red cell aplasia (PRCA) associated with a formulation change of a specific epoetin product reported in 2002 [12–14]. Equivalent therapeutic efficiency with the reference product must be demonstrated in at least two randomized, parallel-group clinical trials, which are preferably double-blind**. The document also states that patients with renal anemia would be the best study population and that after an initial titration phase the comparative phase should be at least 12 weeks, followed by a maintenance study of at least three months. Therapeutic equivalence must be demonstrated for both predialysis and haemodialysis patients with chronic kidney disease, and by both the intravenous and subcutaneous routes of administration. Clinical trials should be adequately powered, and at least 12 months of immunogenicity data should be provided.

The Retacrit® case

Five biosimilar epoetins that are manufactured by two companies have been approved. Abseamed®, Binocrit®, and Epoetin alfa Hexal® are epoetin alfa products and are biosimilar versions of the reference product Eprex®, all produced by Rentschler Biotechnologie GmbH but marketed by three different companies [15–17].

Two additional biosimilar versions of Eprex®, Retacrit® and Silapo® are manufactured by Norbitec GmbH [18,19]. Although this biosimilar manufacturer also used Eprex® as a reference product, the international non-proprietary name (INN) for these products is epoetin zeta rather than epoetin alfa.

The EMEA/CHMP discusses in a European Public Assessment Report (EPAR) the scientific arguments for their positive opinion to grant a marketing authorization [20].

I will discuss here the way the EMEA/CHMP has evaluated Retacrit® that I will refer to as epoetin zeta. The reason for choosing this EPAR is the problems that were apparently encountered when assessing the clinical equivalence. Whenever possible and feasible I will compare the data on epoetin zeta with the data concerning the biosimilar alpha. The problem when comparing different EPAR's is that these documents are not exhaustive and standardized descriptions of the evaluation by the EMEA/CHMP and many aspects are discussed differently. So one detail discussed extensively in the EPAR of epoetin zeta may be completely absent in the EPAR of the biosimilar epoetin alpha.

For the quality comparability exercise, epoetin zeta was compared to epoetin alfa. In the comparability study focused on the protein backbone, the data obtained demonstrated comparability between the two epoetin products. With respect to the glycan moieties, the overall range of structures was found to be comparable. The amount of glycoforms without an O-glycan chain was, however, slightly higher for epoetin zeta as compared with epoetin alfa. On the contrary, the amounts of undesired variants of sialic acid, N-glycolyl neuraminic acid and O-acetyl neuraminic acid were higher in the reference product as compared with epoetin zeta. This difference in glycosylation comes as no surprise because glycosylation is highly dependent on the process used to produce it. Also the EPAR of the other biosimilar, epoetin alpha, mentions glycosylation differences with the original product.

Comparison of the purity and *in vivo* bioactivity did not reveal any remarkable difference. In terms of quality, the overall comparability of Retacrit® and Eprex® was considered demonstrated. The biological activity of Retacrit® was tested in a normocythaemic mouse assay as described in the European Pharmacopoeia. The activity was compared to Eprex® and the international standard EPO-BRP#2 provided by the European Directorate for the Quality of Medicines. The assay quantifies reticulocytes by fluorescence activated cell sorting of blood drawn four days after one subcutaneous administration to female B6D2F1 mice. The relative potency should be between 0.80 and 1.25. The International standard EPO-BRP#2 and two batches of Retacrit® drug product were within this relative potency relative to Eprex®.

In the EPAR, two pharmacokinetic studies in healthy volunteers to demonstrate similar PK profiles are discussed. One was a two-

period crossover study in 24 health volunteers comparing the profiles of Retacrit® and Eprex® after a single IV dose.

The other was a three-period crossover trial in 48 healthy volunteers comparing the pharmacokinetic profiles after a single SC dose of Eprex® as well after a single dose of SC and IV administered Retacrit®.

Epoetin plasma concentrations were analyzed using a validated modified double sandwich ELISA** used.

The study results suggested overavailability of Eprex® compared to Retacrit®. According to the certificates of analysis of the test and the reference drug, a significant difference was present between the batches of both products regarding the total protein content

Owing to this reason, a correction of epoetin serum concentrations based on the protein content of the used batches was performed in both studies which resulted in pharmacokinetic parameters well within the post hoc defined equivalence margins.

Retacrit® was evaluated in two pivotal trials, a correction phase study and a maintenance study. The first study involved 305 patients in the Retacrit® group and 304 patients in the Eprex group of which respectively 275 and 272 patients completed the study. The maintenance study involved 313 patients in a crossover design and 282 of these were available for full analysis. A comparative trial in renal anemia patients using the SC routeof administration was not possible owing to the temporary contraindication of Eprex® at that time.

The study results indicate that both products can control Hb levels to the same extent, but the required doses of Retacrit® needed to achieve control were outside the pre-defined equivalence margin. The CHMP/EMEA subsequently accepted the clinical equivalence because the predefined equivalence margin of 14 IU/kg/week had been wrongly included in the protocol and should be corrected to 45 IU/kg/week (i.e. 15 IU/kg given three times weekly and not only once weekly). Since the background variability in Hb is high in this patient population, even in 'stable' patients on stable epoetin doses, the chosen limit of 1 g/dL for definition of clinical relevance appeared acceptable.

In an uncontrolled study, the efficacy and safety of the biosimilar epoetin in the treatment of chemotherapy associated anemia was evaluated in 216 patients, of which 208 were available for complete evaluation. The study was not, however, designed to establish comparable efficacy for the SC route of administration.

Overall, there was no significant difference between the treatment groups for the incidence or type of adverse events. Retacrit® revealed a safety profile similar to that of the innovator comparator Eprex®.

How bioequivalent is Retacrit®?

The data concerning the potency and specific activity of Retacrit® as described in the EPAR are confusing. The potency test performed in mice indicated an equivalent potency that is normally expressed as IU/ml. According to statements in the EPAR the specific activity expressed in IU/ug protein of Retacrit® and Eprex® are remarkably similar.

In the pharmacokinetic studies in volunteers, who were dosed according to the labeled biological activity, a correction factor was, however, introduced because the protein content of Eprex® was higher than Retacrit®. The pharmacokinetics analysis was based on an immunoassay measuring mass, which justifies a normalization based on the mass the volunteer received. Assuming an equivalent potency and specific activity the protein concentrations of both products should, however, have been the same.

TABLE 1

The potency of Eprex compared with Retacrit in the normocythaemic mouse assay.

	Eprex®	Retacrit®
Nominal (labeled) dose	10 000 IU	10 000 IU
Amount of Epoetin (μg/ml)	113 (±8)	89(±17)
Biological activity (IU/ml)	12 884 (10 860–15 285)	11 016 (8942–13 571)

The biological activity was tested blindly by the National Institute of Biological Standards and control in the mouse assay as described in the text.

In the clinical efficacy studies Retacrit® also appeared to have a lower activity than Eprex®, but consequently clinical equivalence was accepted by the EMEA once the correct clinical equivalence margin was used.

We recently conducted an extensive quality and potency studies of the biosimilars on the European market compared with Eprex® and Dynepo® (epoetin delta). The details of this study will be published elsewhere. In Table 1 the absolute potency of epoetin content of Eprex® and Retacrit® are listed. In the 10 000 IU presentation we found the potency of Eprex to be 10% higher than labeled upon repeated testing. The potency of epoetin zeta (Retacrit® is as labeled. So the lower activity of epoetin zeta compared with Eprex® seems to be caused by a higher potency than nominal value of Eprex®. Why this difference was not encountered when biosimilar alpha was compared with the same innovator product is unclear. The developers of this biosimilar may have corrected the dosage based on potency testing in their clinical trials. The company marketing) Retacrit® kindly provided me with the data on the different batches used during clinical development (Table 2) [21]. Although there were differences in the bioactivity in the batches, Eprex® batches were found on average to have 9% higher bioactivity than the labeled strength and epoetin zeta batches have 1% higher bioactivity than the labeled strength, all batches remained within the limits defined by the European Pharmacopeia, namely 80–125% (with error limits of 64–156%). On the contrary, the average specific

TABLE 2

Bioactivity of different Retacrit® and Eprex® batches used in the clinical trials (data provided by Hospira)

Product	Batch #	Strength/unit (IU)	Bioactivity (IU/unit)
Retacrit®	001	1000	1030
	002	2000	1840
	003	2000	2060
	004	2000	2200
	005	1000	1021
	006	2000	2104
	007	1000	934
	008	2000	2052
	009	1000	940
	010	2000	2190
	011	2000	2070
Eprex®	001	1000	980
	002	2000	2300
	003	1000	1016
	004	2000	2151

bioactivity of the two proteins was similar (130.80 for Retacrit® vs.130.75 units/μg for Eprex®).

So the apparently reduced bioactivity of epoetin zeta is caused by a too high activity of Eprex® in the presentations tested. These data show that the batch-to-batch difference seen in bioactivity could be due to the current obligatory mouse assay for potency testing is which is too imprecise for bioequivalence studies.

Conclusions

The classical paradigm of bioequivalence cannot be applied to protein drugs. Therefore the EMEA/CHMP has introduced the concept of biosimilarity. To be considered biosimilar, equivalence in quality, efficacy and safety has to be shown with the original product. As the example of Retacrit® shows there are a number of *caveats* concerning bioequivalence studies. There is a batch-to-batch variation in potency in both the biosimilar and original product and therefore in future biosimilar studies, more extensive testing should be done of the batches used.

Also, for the bioequivalence studies the dosing of subjects should be based on the potency found and not based on nominal values. Another important lesson is that the testing as mandated by the Pharmacopeia is sometimes not meeting the standards necessary to establish the equivalence of protein products.

Although differences were seen for both products, in clinical trials there was no clinically significant difference seen between the two products to treat patients to correct anemia and maintain target haemoglobin.

Every biological product is different and treating physicians and healthcare professionals should interchange biologicals following careful analysis of the supporting data [22].

Conflict of interest

Conflicts of interest statements concerning the specific products discussed in this paper.

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