Comparative testing and pharmacovigilance of biosimilars

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Abstract
Unlike traditional generic pharmaceuticals, biosimilars (also called ‘follow-on biopharmaceuticals’ in the USA) aim to copy a complex recombinant, three-dimensional protein structure with high molecular weight. Small changes in the manufacturing process can alter the product’s effect and safety. According to the guidelines of the European Agency for the Evaluation of Medicinal products (EMEA), extensive comparability testing will be required to demonstrate that the biosimilar has a comparable profile in terms of quality, safety and efficacy as the reference product. Various analytical assays are available to compare physicochemical and biological properties between production batches of a potentially similar biopharmaceutical (comparability) and in comparison with a reference product (similarity). It is important to recognize the limits of existing assays so that the results can be accurately interpreted for market authorization.

This article examines the quality and limits of such analytical methods. The analytical tests to demonstrate comparability and similarity of a biosimilar product to a reference drug with respect to protein content, activity, physiochemical integrity, stability, impurities and additives, as well as immunogenicity are discussed. Although several assays are available, reliable tests for safety and efficacy still require development. Furthermore, international standards are missing and materials and methods differ from laboratories making the comparison of results very difficult. Clinical trials and post-authorization pharmacovigilance are essential to guarantee the product’s safety and efficacy over time. Pharmacovigilance, as part of a comprehensive risk management programme, will need to include regular testing for consistent manufacturing of the drug.

Keywords: assays; biopharmaceuticals; biosimilars; comparative testing; pharmacovigilance

Introduction

As patents of first generation biopharmaceuticals derived from recombinant DNA are expiring, the development of ‘biosimilars’ is increasing. Follow-on biopharmaceuticals aim to copy complex recombinant, three-dimensional proteins with high molecular weight. Their market authorization procedure cannot be based on traditional generics of pharmaceuticals, as the activity of biopharmaceuticals depend on a multitude of factors [1,2].

Guaranteeing consistency in the production of these agents has already proved difficult [3]. Incidences such as the increased occurrence of pure red cell aplasia (PRCA) cases in 1998 demonstrated how one small change in the manufacturing process can alter the product’s characteristics [4]. Such complexity means that requirements for marketing authorization of biosimilar products cannot be the same as for low-molecular weight generic drugs.

Therefore, preliminary guidelines for pre- and post-market authorization of biosimilar products from the European Agency for the Evaluation of Medicinal Products (EMEA) demand extensive testing to ensure that the biosimilar has a similar quality, safety and efficacy profile as its reference product [5].

Various analytical tests are available to analyse the physicochemical properties (such as weight, density and stability) and biological properties (such as activity and immunogenicity) of biosimilars. These assays are necessary to test the similarity and comparability of a biosimilar against the innovator drug. It is important to recognize the limitations of available assays for such testing and encourages careful interpretation of results to ensure continued safety and efficacy in the target populations. The qualities of analytical assays have a central role in the decision-making for marketing authorization of biosimilar products. This study explores the quality and limitations of various
analytical methods currently used in comparing biosimilars with their reference products to support the development of comprehensive guidelines, regulations and the safe use of biosimilars in practice.

**Analysis of biosimilar products**

**Content**

Differences in the molecular weight of similar molecules indicate potential structural variations and, therefore, possible disparity in activity or other unknown outcomes. While chemically synthesized compounds of up to 1000 Da can be accurately estimated within 1/100 Da, the molecular weight of biopharmaceuticals (up to 145 000 Da) can vary by 1000 Da due to the heterogeneity of production processes and resulting products within a single manufacturing process [6,7]. Product-specific enzyme-linked immunosorbent assays (ELISAs) and size exclusion chromatography are used to determine such qualities like quantity, molecular weight or distribution of biological molecules. These assays, however, are limited by the requirement for high-quality reference material which is often not readily available in sufficient quantities and are inadequate to fully characterize complex proteins. In addition, quantifying the clinical significance of such results is difficult. Therefore, all detected differences should be interpreted as potential safety risks.

**Biological activity**

Even if the weight and density of a biosimilar is similar to its reference drug, its activity and efficacy may vary considerably. Biological properties may be measured in vitro or in vivo depending on the nature of the product. For activities such as binding of an antibody to a specific target, in vitro assays are informative options. Testing of 12 batches of epoetins from five manufacturers showed variations in the potencies from 80 to 125% from those stated on the labels (95% confidence interval: 64–156%) [8]. Different potencies even between product samples from the same manufacturer were demonstrated. However, in vivo assays remain the most relevant means to compare biological activities between products.

**Physicochemical integrity**

The glycosylation profile of an active substance and potential impurities from aggregates and degraded products can also affect the activity of a biologically manufactured drug. The purity of a product can be tested by various methods including sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), reverse phase high performance liquid chromatography (RP-HPLC), size exclusion chromatography, mass spectroscopy or carbohydrate analysis (important for glycoproteins in general). These methods, however, may be limited by the availability of sufficiently large samples for processing and sample preparation is particularly labour intensive.

Nevertheless, these assays have been useful for detecting differences in physicochemical integrity and glycosylation patterns between similar products [9,10]. This clearly demonstrates the difficulty of creating a ‘new’ biosimilar and producing it consistently.

**Stability**

The stability of a product is generally highly dependent on its storage conditions, which must be clearly defined according to the product’s characteristics. A biopharmaceutical from the same manufacturer may also degrade at different speed and at different conditions due to inadequate control of the production process.

The stability of a molecule and its degradation pathway can be studied by exposing it to increased temperature. Gel-electrophoresis of an unstable product will show various bands apart from the principal molecule, indicating a higher tendency to form aggregates. Decreased stability in comparison with the reference product may either be due to an unstable formulation or an active ingredient.

**Formulation**

The formulation of a biopharmaceutical is as important as the active ingredient. Additives (e.g. human serum albumin (HSA), polysorbates, glycine, amino acids, calcium chloride and urea) are routinely used to stabilize protein drugs [11]. However, the additives may also influence a drug’s safety profile. Initial analyses of a biosimilar’s active ingredient may indicate acceptable similarity to the reference molecule, but the final product formulation may reveal potential risk factors.

Absorption spectra of different formulations of similar products can reveal differences between them and identify chemicals and impurities that may have leached into the formulation [12]. Such leachates or the possible formation of EPO-containing micelles due to the high concentration of polysorbate 80 [13] and other unknown causes may have been responsible for the increased occurrence of PRCA cases after the Eprex® formulation had changed [14,15].

**Analysis of immunogenic responses**

The most important safety issue for biosimilars is immunogenicity. Comparing products between manufacturing batches and with an established reference product during pre-authorization studies is necessary. However, sensitivity and specificity of assays for testing immunogenic responses may still be insufficient to predict rare cases of immunogenicity. Also, the lack of standardization and validation of methods,
but not substitute clinical trials. Rare but severe outcomes with long incubation periods may not be detected by analytical tests or clinical trials before authorization.

For this reason, post-authorization pharmacovigilance will be critical to reduce the risk of developing unwanted outcomes such as immunogenicity.

**Pharmacovigilance**

Pharmacovigilance relates to the detection, assessment, understanding and prevention of adverse effects after a product is available on the market. Although close pharmacovigilance is a voluntary post-marketing process, it will be in the interest of the manufacturer to guarantee the quality, safety and efficacy of the biosimilar over time. The EMEA guidelines state that a comprehensive pharmacovigilance plan should be sent to the authorities together with the data package and such a plan should be established at the time of approval of the product [20]. Nevertheless, existing pharmacovigilance strategies were slow in detecting and reacting to the rise in PRCA cases. This questions the appropriateness of the current system for the continuous post-marketing evaluation of biosimilars. Furthermore, a minimum number of cases of this rare disorder are needed to establish its association with a specific treatment. Predictive models to overcome this problem vary in accuracy and will need improvement to serve as decision criteria.

A comprehensive product profile is the basis for entering the market. Databases from clinical practice with detailed information and regular evaluation complements the safety evaluation.

A complete risk management programme is needed which includes regular pre- and post-authorization comparative testing as well as careful pharmacovigilance. The manufacturing process must be carefully monitored to ensure comparability between production batches. If a difference in the manufactured product is detected, additional investigations may be necessary, which may include clinical proof of unchanged safety and efficacy profile. It will also be essential to define ‘who’ and ‘when’ at the right place and time to do ‘what’ and ‘how’. This is especially important for the risk management component of the programme that needs to guarantee immediate reaction in case of rising numbers of patient disorders with suspected relation to the biosimilar product.

**Conclusion**

In summary, biosimilars need to be tested comprehensively within the production process and is always in comparison with an appropriate reference product. Although a variety of assays are available, they may not be sufficient to predict consistent safety and efficacy of a biosimilar product. Standardization and
validation of assays and the presentation of data is crucial for future credible testing of biosimilars. Nevertheless, only clinical studies and post-authorisation pharmacovigilance will provide essential evidence for product quality.

Conflict of interest statements. F.L. is currently conducting research sponsored by Amgen, Roche, Shire and is also a member of advisory boards for those companies. S.R. has received funding for research, speakers bureau and for attendance at scientific meetings in addition to consultancy fees from Amgen, Janssen Cilag and Roche. He is a current standing member of the Australia and New Zealand Society of Nephrology (ANZSN) Pharmaceutical sub-committee.

References