ISSN: 0976-822X

Available online on www.ijcpr.com

International Journal of Current Pharmaceutical Review and Research 2022; 14(3); 16-21

Original Review Article

INSIGHTS ON IDENTIFICATION OF BIOSIMILAR DRUGS USING VARIOUS ANALYTICAL TECHNIQUES

R. Swetha Sri*, G. Srija, K. Bhavya Sri, M. Sumakanth

Department of pharmaceutical analysis, RBVRR women's college of pharmacy, Barkatpura, Hyderabad

Received: 15-09-2022 / Revised: 16-09-2022 / Accepted: 18-09-2022
Corresponding author: R. Swetha Sri
Conflict of interest: Nil

Abstract

A biosimilar drug is a biological product that is approved based on demonstrating very similar to an approved biological product, known as reference biologic, for which there are no clinically meaningful differences in terms of safety, potency and purity. Biosimilar drugs have the potential to enhance treatment accessibility. They have the same strength and dosage as that of the reference product. The analytical biosimilar studies are conducted to demonstrate a highly similar profile concerning variations in biosimilar product's critical quality attributes (CQAs). To achieve a successful approval of a biosimilar drug, establishing analytical and clinical biosimilarity is required. Approximately 90-95 drugs have gained approval so far in our country where studies are prevalent to increase the number and be handy for available to all classes of population. Byooviz(ranibizumab) having lucentis as its reference product was approved in the year sep 2021, whereas semglee(Insulin glargine)got its entry into the market in July 2021.The "Purple book" contains information including online database about FDA-licensed biological products which are regulated by CDER and CBER.Various chromatographic and spectroscopic techniques are used for biosimilar assessment which includes mass spectroscopy, reversed-phase chromatography, ion exchange chromatography, hydrophobic chromatography, hydrophilic interaction liquid chromatography, size exclusion chromatography, x-ray crystallography, multidimensional NMR spectroscopy, FTIR, fluorescence spectroscopy and also hyphenated techniques such as 2D-LC/MS, HILIC/MS.

Keywords: Biosimilars, reference product, Generic drugs, CQAs, 2D-LC/MS,HILIC/MS

INTRODUCTION

Biosimilar drugs are the drugs that are the generic versions of approved biological products. The development of biosimilar is saving time and resources by avoiding unnecessary duplication of clinical trials. Biosimilars are produced from biological processes, whereas generic drugs and obtained by chemical synthesis. Generic drugs contain identical medicinal ingredients to their reference products. However, biosimilars and biologics and very similar but not identical [1]. Both generic and biosimilars undergo a thorough but shortened FDA review process compared to their reference products. A few examples of the biosimilars and their reference products that have been approved and being marketed in different parts of the world are as listed in Table 1.

Table 1: Information regarding few biosimilar drugs, its reference product and active ingredient.

BIOSIMILAR	REFERENCE PRODUCT	ACTIVE INGREDIENT
Omnitrope [2]	Genotropin	Somatropin
Zarxio [3]	Neupogen	Filgrastim
Renflexis [4]	Remicade	Infliximab
Amjevita [5]	Humira	Adalimumab
Herzuma, Kanjinti, Ogivri	Herceptin	Trastuzumab

2. Legislation of biosimilars in various countries:

2.1 INDIA:

The legislation in India is given by the "Guidelines on Similar Biologics," by the Central Drugs Standard Control Organization (CDSCO) and the Department of Biotechnology (DBT) for a Similar Biologic claiming to be similar to an authorized biologic. A Similar Biologic product is similar in terms of quality, efficacy and safety to an approved Reference Biologic product based on comparability. The Similar Biologics are regulated as per the Drugs and Cosmetics Act 1940 and rules 1945 and Rules for the manufacture, use, import, export and storage of hazardous microorganisms/ genetically engineered organisms or cells 1989 (Rules 1989) notified under the Environment (Protection) Act 1986. Various applicable guidelines are Recombinant DNA Safety Guidelines 1990, Guidelines for generating preclinical and clinical data for rDNA vaccines, diagnostics and other biologics 1999, CDSCO guidance for industry 2008, Guidelines and Handbook for Institutional Biosafety Committees (IBCs) 2011 and Guidelines on Similar Biologics: Regulatory Requirements for Marketing Authorization in India 2012. According to the "Guidelines on Similar Biologics" any product can be considered as a Similar Biologic only if it is proven to be similar using extensive quality characterization against Reference Biologic. The demonstration of similarity depends upon detailed and comprehensive product characterization, preclinical and clinical studies carried out compared to a Reference Biologic. There are nearly 100 biosimilars that sought approval in India, with at least 50 in the market. The first biosimilar to get approval and be marketed was for hepatitis B in the year 2000, known as Biovac-B for treating hepatitis B manufactured by Wockhardt.

2.2 EUROPEAN UNION:

For the Biosimilars to be approved for marketing in the EU, the European Medical Agency's (EMA) scientific Committee on Human Medical Products (the CHMP), as well as EU experts on biological medicines (Biologics Working Party) and specialists in biosimilars (Biosimilars Working Party), evaluate the data provided by the manufacturers/company. The review by EMA results in scientific opinion, which is then sent to the European Commission for granting EU-wide marketing authorization. The data provided must demonstrate high similarity in quality, efficacy and safety of the active substance of the biosimilar with that of the reference product. The high similarity can be achieved by performing comprehensive comparability studies with reference products. In 2006, Omnitrope was the first biosimilar to seek approval for marketing in the EU.

2.3 UNITED STATES OF AMERICA:

A statutory provision referred to as Biologics Price Committee and Innovation Act 2009 gives an abbreviated approved pathway for biological products that are demonstrated to be highly similar to an FDA-approved biological product. There are two review committees, the Center for Drug Research and Evaluation (CDER) Biosimilar Review Committee and the Center for Biologics Evaluation and Research (CBER) Biosimilar Review Committee which address the product-

specific review and issues relating to scientific methodology. Under BPCI Act, the sponsor seeks approval of a Biosimilar product under section 351(k) of the PHS Act. Zarxio by Sandoz, a biosimilar to Neupogen, was the first biosimilar to be approved for marketing in the USA.

2.4 CANADA:

Here, in Canada, Biosimilars are regulated as new drugs under the Food and Drugs Act and the Food and Drug Regulations. The biosimilars seek approval for sale after a thorough comparison to a reference biologic. Thus, biosimilar manufacturers have to provide information to Health Canada which manifests the similarity of their biosimilar with the reference biologic using comparative studies based on a step-wise approach, initially with structural and functional studies and then continuing with the human clinical studies. Before comparative studies, one must independently exhibit the quality of the biosimilar in order to seek marketing approval. Byooviz, a biosimilar of Lucentis (ranibizumab), was the first biosimilar to get marketing approval in Canada.

3. Analytical techniques used in the identification of biosimilars:

CQAs are performed using analytical platforms like established biophysical tools and new technologies. Over the years, some orthogonal methods and procedures for assessing CQAs have been adopted for routine biosimilarity studies. The analysis of primary, secondary and tertiary structures are performed using different analytical methods.

3.1 Analysis of primary structure:

3.1.1 Mass Spectrometry: These days, mass spectrometry is the most widely used technique in characterizing the primary structure of proteins. The intact mass analysis provides essential information that can verify the amino acid sequence using an observed protein mass and gives the protein's identity and relative abundance of any resolved isoforms.

3.1.2 Glycan Analysis

Protein glycosylation is a post-translational modification that directly affects the protein's function. The presence of glycans modifies the structure and function. Most biosimilars are glycosylated, as more than 50% of human proteins are glycosylated. Regulatory agencies consider glycosylation a Critical Quality Attribute (CQA); therefore, it is monitored closely during manufacturing and stability studies.

3.1.3 Reversed-phase chromatography (RPC)

Reversed-phase chromatography is used for protein identification, impurity profiling, intact and subunit mass measurements, and peptide map analyses. It is also used to separate labeled released glycans. It offers an orthogonal separation to HILIC in glycan analyses. It is one of the most widely used techniques for separating proteins, nucleic acids and peptides. Hence, the use of volatile mobile phases enables coupling to mass spectrometry.

3.1.4 Hydrophobic interaction chromatography

The hydrophobic interaction chromatography (HIC) technique is similar to RPC. The stationary phases used for HIC are usually low-density and moderately hydrophobic ligands (propyl and phenyl). Usually, a decreasing salt gradient is applied in the presence of organic content. Salts are incompatible with mass spectrometry, but newer stationary phases, like polypentyl to polydecyl, provide good separation even when ammonium acetate is used, making the method applicable to mass spectrometric detection. Hydrophobic interaction chromatography helps monitor methionine or tryptophan oxidation, asparagine isomerization, and serine

phosphorylation. It is a non-destructive analytical method coupled with mass spectrometry for top-down analyses. HIC is a powerful analytical technique for analyzing antibody-drug conjugates and is routinely used to determine the drug to antibody ratio.

3.1.5 Ion-exchange chromatography (IEX):

Ion-exchange chromatography is used to analyze charge variants based on the charged groups that are constantly attached to the surface of column packing. These charged groups connect with the counter ions provided by the buffer or salt in the mobile phase, which are of opposite charge. These sample ions compete with counter ions for access to the ion exchange sites. Retention time results from ionic interaction between the sample and the ion exchange resin. Ions with less affinity towards the ion-exchange resin will elute first, and ions with more affinity will elute later. Ion exchange chromatography is divided into two categories: cationic and anionic exchange chromatography. Cationic exchangers possess negatively charged groups that attract positively charged analytes, whereas anionic exchangers have positively-charged groups that attract negatively charged molecules. Most protein chromatography is done using IEX. IEX has a wide range of applications in analyzing charge variants of proteins. Since a change in a single amino acid or lack of a single amino acid may change the isoelectric point (pI) of the whole protein, this method is susceptible to detecting differences in the primary structure. Truncations are post-translational modifications that alter the overall charge of the protein.

3.1.6 Size-exclusion chromatography:

Size-exclusion chromatography (SEC) analyzes proteins of various sizes, particularly aggregates, fragments of proteins and subunits. The pore size of the stationary phase determines the applicable molecular weight range of a size exclusion column. Size exclusion chromatography does not provide a high-resolution method and is not capable of separating analytes of similar size. The stationary phase comprises hydrophilic silica, polymer-based, or porous hybrid organic/inorganic particles (BEH columns). The mechanical strength of BEH particles reduces particle size to 1.7 mm, thereby offering more chromatographic efficiency using UPLC instrumentation. Organic modifiers and volatile buffers are being introduced into SEC methods to make them suitable for mass spectrometry. SEC-HPLC is mainly used in assessing high molecular weight (HMW) aggregates and monomeric purity of antibody therapeutics, a critical quality attribute (CQA) monitored for a biosimilar.

3.1.7 Hydrophilic interaction liquid chromatography (HILIC):

Hydrophilic interaction liquid chromatography is a separation technique that uses a polar stationary phase and solvents similar to that of reversed phase chromatography (e.g., water, methanol, acetonitrile, volatile buffers and modifiers). This technique is directly coupled to mass spectrometers as all the mobile phases are suited for the MS. As HILIC stationary phases are polar, the technique is well-suited for analyzing polar molecules with limited or no retention on reversed phases like C8 and C18. In cases of biosimilar characterization, the HILIC method is used for glycan analysis. As the initial conditions are highly organic (>60%), the HILIC method can only be used to separate proteins that are soluble at such high organic content. Since the separation is based on glycans' size and structure, if those are still attached to the respective peptides, subunits, and even intact proteins. It is possible to separate the peptides and proteins. The Glycopeptides and glycoproteins can be separated in HILIC using wide pore-size columns (300

– 400 Å instead of 130 Å pore size (glycan columns)).

3.2 Analysis of secondary and tertiary structures

As all the optical spectroscopy method relies on a reporter moiety that relates to a structural characteristic of the protein, combining these methods provides complementary information on the short segments of proteins, which are spatially close but are not necessarily adjacent in sequence for biosimilar comparability. Circular Dichroism is a helpful tool in the study of the secondary and tertiary structure of a protein. The Far UV Circular Dichroism (190 to 260 nm) spectrum of the proteins can provide the fractional evaluation of secondary structure features such as the alpha helix, beta-sheet, and random coil conformation. The Near UV Circular Dichroism (250 to 300 nm) spectrum of a protein provides information about the protein's tertiary structure and fold state. The spectrum within this region cannot be given to a particular tertiary structure. The CD signal from aromatic amino acids like phenylalanine (250 to 270 nm) tyrosine (270 to 290 nm), and tryptophan (280 to 300 nm) shows strong signals when present in a definite rigid structure, indicating that the protein is folded into a definite structure. The broad weak signal from disulfide bonds (250 to 260 nm) can be beneficial in analyzing the folding state of monoclonal antibodies. In combination with FTIR and antibody array mapping, circular dichroism spectroscopy is used to characterize infliximab's biosimilar for FDA approval.

4. Some practical considerations:

a) Comprehensive characterization of the heterogeneity of adalimumab via charge variant analysis hyphenated on-line to native high-resolution Orbitrap mass spectrometry.

b) Comparison of originator and biosimilar therapeutic monoclonal antibodies using comprehensive two-dimensional liquid chromatography coupled with time-of-flight mass spectrometry.

c) Rapidly comparing a candidate biosimilar to an innovator monoclonal antibody (trastuzumab) with advanced liquid chromatography and mass spectrometry technologies.

d)Hydrophilic Interaction Chromatography Hyphenated with Mass Spectrometry: A Powerful Analytical Tool for the Comparison of Originator and Biosimilar Therapeutic Monoclonal Antibodies (Remicade/Remsina/Inflectra, Herceptin/Trastuzumab B, and Erbitux/Cetuximab B) at the Middle-up Level of Analysis.

e) FTIR spectroscopy as an analytical tool to compare glycosylation in therapeutic monoclonal antibodies.

f) UHP-SEC is a powerful tool to show the similarity between the innovator drug (Humira) and its biosimilar.

ACKNOWLEDGMENT:

I want to acknowledge our beloved principal Prof. M. Sumakanth and the Faculty of the department of pharmaceutical analysis for giving me this opportunity.

References

1. Patel, P. K., King, C. R., & Feldman, S. R. (2015). Biologics and biosimilars. Journal of Dermatological Treatment, 26(4), 299–302.
2. Omnitrope. (2021, November 16). European Medicines Agency.
3. FDA Approves First Biosimilar, Zarxio. (2015). Cancer Discovery, 5(5), 460.
4. News-Medical.net. (n.d.). Renflexis.
5. Office of the Commissioner. (2016, September 23). FDA approves Amjevita, a biosimilar to Humira. U.S. Food and Drug Administration.

6. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6394151/
7. Https://ibkp.dbtindia.gov.in/DBT_Content_Test/CMS/Guidelines/20181115140059519_Guidelines-on-Similar-Biologics-2016.pdf
8. Ghia, C., Rambhad, G., & Rao, U. (2016). Biologics and Biosimilars: Differences and Clinical Implications. British Journal of Medicine and Medical Research, 12(7), 1–7.
9. www.fda.gov/drugs/guidance-compliance- regulatory-information/implementation-biologics- price-competition-and-innovation-act-2009
10. www.canada.ca /en/health-canada/services/drugs-health-products/ biologics-radiopharmaceuticals-genetic-therapies /biosimilar-biologic-drugs.html
11. Günther, H. (1995). NMR Spectroscopy: Basic Principles, Concepts, and Applications in Chemistry. Wiley Handbook of Pharmaceutical Analysis. (2001). Ahuja.
12. Perry G. Wang, Weixuan He. (2011). Hydrophilic interaction liquid chromatography and advanced applications. CRC press.
13. Comprehensive characterisation of the heterogeneity of adalimumab via charge variant analysis hyphenated on-line to native high resolution Orbitrap mass spectrometry. (2018, November 11). Taylor & Francis.
14. Pisupati, K., Tian, Y., Okbazghi, S., Benet, A., Ackermann, R., Ford, M., Saveliev, S., Hosfield, C. M., Urh, M., Carlson, E., Becker, C., Tolbert, T. J., Schwendeman, S. P., Ruotolo, B. T., & Schwendeman, A. (2017). A Multidimensional Analytical Comparison of Remicade and the Biosimilar Remsima. Analytical Chemistry, 89(9), 4838–4846.
15. Comparison of originator and biosimilar therapeutic monoclonal antibodies using comprehensive two-dimensional liquid chromatography coupled with time-of-flight mass spectrometry. (2016, 24 July). Taylor & Francis.
16. D'Atri, V., Fekete, S., Beck, A., Lauber, M., & Guillarme, D. (2017). Hydrophilic Interaction Chromatography Hyphenated with Mass Spectrometry: A Powerful Analytical Tool for the Comparison of Originator and Biosimilar Therapeutic Monoclonal Antibodies at the Middle-up Level of Analysis.
17. FTIR spectroscopy as an analytical tool to compare glycosylation in therapeutic monoclonal antibodies. (2020, 22 may). ScienceDirect
18. Sorensen, M., Harmes, D. C., Stoll, D. R., Staples, G. O., Fekete, S., Guillarme, D., & Beck, A. (2016). Comparison of originator and biosimilar therapeutic monoclonal antibodies using comprehensive two-dimensional liquid chromatography coupled with time-of-flight mass spectrometry. mAbs, 8(7), 1224–1234.