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REVIEW PAPER

Biosimilars: recent developments

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Abstract Biopharmaceuticals are recombinant protein drugs which are produced by biotechnology. The availability of such molecules has revolutionised the way we treat many diseases. However, the patents for many originator biopharmaceuticals are expiring, and a new generation of follow-on molecules, termed ''biosimilars'', are under development. Health care providers perceive biosimilars to be cheap replacements for originator drugs such as recombinant human erythropoietin and human growth hormone. However, concerns have been raised about the comparability of biosimilars with originator products especially in light of the complex manufacturing process required to produce biopharmaceuticals. The complexity of protein molecules renders it impossible to produce identical copies; this in turn raises questions on the safety of follow-on biosimilar products, particularly with respect to immunogenicity. This review briefly outlines the process of biopharmaceutical production, potential problems that can arise from their long-term use in patients, and the issues facing regulatory bodies as they look to institute guidelines for new biosimilar molecules.

Keywords Biosimilars · Biopharmaceuticals · EMEA · EPO · Immunogenicity · PRCA

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1 Biosimilars: recent developments

Biopharmaceuticals have revolutionised treatment options for several diseases, including anaemia associated with renal dysfunction. The advent of recombinant human (rh)-erythropoietin (EPO) has changed the way we treat and manage patients with renal disease, enabling us to minimise the risks associated with blood transfusions [1]. For the purposes of this review, the term ''biopharmaceutical'' comprises recombinant protein drugs which are produced by biotechnology. ''Originator'' refers to the initial biopharmaceutical product approved for market release after successfully meeting all of the safety and efficacy tests required of a new drug.

Currently, many originator biopharmaceutical product patents are approaching expiration. In their wake is a new generation of molecules, termed ''biosimilars''. Biosimilars are follow-on versions of originator biopharmaceuticals, claimed by their manufacturers to be similar to the tried-and-tested originator products. Health

care providers are eyeing these new molecules as potential cheaper alternatives to originator biopharmaceuticals. In reality, however, the situation is more complex: a slightly lower market price is not the main factor to be considered when faced with the choice of biosimilars versus originator drugs. Furthermore, the clinical benefits of biosimilars need to be carefully evaluated.

A healthy dose of caution should accompany the emergence of biosimilars as they cannot be brought to market through the same procedure applied to generic small molecule drugs because they are not identical to the original products. The characteristics of biopharmaceuticals that distinguish them from synthetic drugs are summarised in Table 1. This review will briefly outline the process of biopharmaceutical production, potential problems that can arise from their long-term use in patients, and the issues facing regulatory bodies as they begin to implement guidelines for new biosimilar molecules. Where, relevant issues relating to rh-EPO products will be discussed.

2 Biopharmaceuticals: the manufacturing challenge

In the past, almost all pharmaceuticals were low molecular weight synthetic compounds, such as statins (HMG CoA reductase inhibitors) and kinase inhibitors [2, 3]. Their relatively simple structures render them easy to synthesise, and identical molecular ''copies'' can be produced. Biopharmaceuticals, however, are much more complex in size and structure. In terms of size alone, proteins can be 100–1000 times larger than synthetic small molecules. Table 2 lists several examples of biopharmaceutical classes. Amongst the successful biopharmaceutical products that are on the market are recombinant human (rh) insulin, growth hormone, EPO and interferon (IFN)-beta. The large size and complex structure of biopharmaceuticals requires their production to involve complex manufacturing and quality control processes that are highly sensitive to modification during the production process and beyond (summarised in Fig. 1) [4].

The production of recombinant protein molecules involves the use of ''biotechnology'', defined as ''all lines of work by which products are produced from raw materials with the aid of living things'' [5]. Unlike synthetic chemical drugs, biopharmaceutical products require the use of living biological host cells for their production. This includes the use of recombinant genetic engineering techniques for cloning of the appropriate genetic sequence into an expression vector, followed by the generation of a host cell expression system and scaling it up for large-scale protein production. The desired protein must then be isolated and purified from the cell culture medium, using purification techniques that maintain the protein's structural and functional integrity. The purified product must then be correctly formulated, to ensure that it retains its biological activity up to patient delivery. The expression of the same genetic construct in different host cell expression systems has a great impact on the final structure of the protein. For example, rh

Table 1 The characteristics of biopharmaceuticals that distinguish them from small molecule drugs (after Schellekens [33])

Biopharmaceuticals versus chemically synthesised small-molecule drugs
◆ Large complicated molecules
◆ Heterogeneity
◆ Produced by genetically modified living cells
◆ Complex mode of action mediated by large surface area
◆ Complicated production and purification process
◆ Relatively unstable

Table 2 Examples of several classes of biopharmaceuticals

Biopharmaceutical class	Example
Blood factors	Factor VIII
	Factor IX
Thrombolytic agents	Tissue plasminogen activator
Hormones	Insulin
	Growth hormone
	Gonadotropins
Haematopoietic growth factors	Erythropoietin
	Colony stimulating factors
IFNs	IFN-alpha, -beta, -gamma
Interleukin-based products	Interleukin-2
Vaccines	Hepatitis B surface antigen

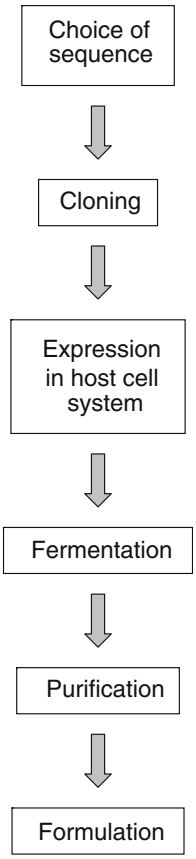

Fig. 1 A summary of the main production steps for biopharmaceuticals

granulocyte colony-stimulating factor (G-CSF) expressed in *E. coli* is non-glycosylated, whereas that expressed in Chinese hamster ovary cells is glycosylated [6]. Although both forms are available for clinical use, the function of the glycosylated moiety is unknown.

Each stage of the production process is an area of intensive research and investigation, from the choice of the expression vector, host cell line, purification protocols, quality control assessments, through to the final product formulation. Any change in the process can have profound effects on the biological activity and safety profile of the final product. One example is modification of a protein's glycosylation pattern. Proteins manufactured in yeast cells contain high levels of mannose sugar groups, rendering them more prone to degradation and thereby decreasing their half-life [7]. The culture conditions in which host cells are cultivated can also affect the glycosylation structure of the expressed protein [8]. In the case of EPO, the serum half-life is dependent upon the presence of four sialylated N-glycans. Increasing the degree of sialylation decreases its clearance rate and increases the in vivo activity [9]. The problem of appropriate glycosylation is just one of the many factors that can be affected by the manufacturing process. Other parameters that can be affected include amino acid structure (oxidation, deamidation), side chains (carbamylation, GSH-adducts), post-translational processing (methylation, acetylation), as well as tertiary and quaternary structure of the protein product.

Even within a single production facility, inconsistencies can occur throughout any step of the process, possibly leading to inter-batch variations. Seemingly undetectable changes to complex three-dimensional protein structure can have profound effects on protein function, such as protein–protein or ligand–receptor interactions. Therefore, the consistency of the protein production process must be carefully monitored, as the slightest change may have significant clinical implications.

With this in mind, it should come as no surprise that products from different manufacturers show marked differences upon analysis [10]. Since the manufacturers of biopharmaceuticals are not obliged to disclose their manufacturing procedures, biosimilars manufacturers have to work out their own manufacturing protocols based upon the final product. When analytic and clinical evaluations of EPO biosimilar products currently marketed outside the US and Europe were conducted, it was revealed that the products' composition differed widely, they exhibited inter-batch variation, and often did not meet self-declared specifications [11, 12]. This variation between products underscores the colossal difficulties in replicating biopharmaceutical proteins.

3 Immunogenicity: a problem to be resolved

All biopharmaceuticals carry the potential risk of initiating an immunogenic response in the patient. The immunogenic potential of biopharmaceuticals

can be influenced by many factors, including the chemical structure of the molecule (including variations in amino acid sequence and glycosylation patterns), physical degradation (such as the formation of aggregates) and chemical decomposition (such as oxidation) [13]. Autoimmune condition (disease profile) or major histocompatibility class type of the patient are also known to play a role in immunogenic response [14]. Although the exact mechanism(s) remain unknown, the route of administration can also have an effect. Changing the route of administration does not eliminate the immunogenic response to a given protein and the risk of immunogenicity progressively increases from local, intravenous, intramuscular, to subcutaneous administration.

In addition to the above factors, downstream processing (such as protein purification) as well as the formulation of a biopharmaceutical product can affect its immunogenic potential. For example, trace amounts of contaminants or impurities have been implicated in antibody development against insulin and growth hormone products [15]. As new and improved techniques for protein purification are developed the problem of impurities may be reduced. Another important factor for consideration is the formulation of the protein product. Product formulation is critical for stabilisation of the protein molecules in order to maintain protein structural integrity (i.e. avoiding the formation of aggregates) and biological activity until delivery to the patient [16]. Patient immunogenic response may be triggered by the administration of inadequately stabilised proteins that have aggregated or denatured [17, 18]. Some molecules, such as the IFNs, have a greater propensity to form aggregates under certain conditions, such as low pH or low denaturant concentrations [19]. IFN-alpha protein aggregates have been found to be significantly more immunogenic than monomers in vivo [20]. The use of a specific assay(s) to detect neutralising antibodies is key to monitoring product quality, along with close surveillance of efficacy in order to immediately detect any increases in antibody production and drops in pharmacological effects.

While the incidence of immunogenic response is generally low as immune tolerance failure is an inherently slow process, it has a relatively high incidence rate in specific cases, such as for IFN-beta [21, 22]. In the case of the IFNs, the presence of neutralising antibodies has negative effects on clinical efficacy and bioavailability [23]. Likewise, a major complication in clotting factor replacement therapy for the treatment of haemophilia is the development of inhibitory antibodies to factor VIII [24]. In rare instances efficacy is inadvertently enhanced, such as for growth hormone treatment in children where it has been found that binding antibodies boost growth hormone efficacy by stabilising the protein [25]. However, this may ultimately have negative consequences for the patient, as precise dose control is lost.

In most instances, neutralising antibodies against a biopharmaceutical could lead to loss of efficacy, and at worse, neutralisation of the native protein. The case of the EPO-α molecule, Eprex® (Johnson & Johnson) illustrates how a small and seemingly inconsequential process alteration can have a dramatic impact on the patient. The use of this molecule for treatment of anaemia in patients with renal dysfunction has been associated with an increased incidence of pure red cell aplasia (PRCA). PRCA is a severe, isolated, non-regenerative, sudden-onset anaemia, characterised by an almost complete cessation of red blood cell production [26]. The increased incidence of PRCA reportedly coincided with the switch to polysorbate 80 from human serum albumin in the product formulation. One theory is that the presence of leachates from uncoated rubber syringe stoppers triggered the immunogenic response [27]. Although the actual cause(s) remains to be proven, the key message is that the outcome of any alteration in the manufacturing process is unpredictable. The occurrence of PRCA demonstrated that serious consequences for the patient can result from even subtle changes in the manufacturing process in established biopharmaceuticals.

4 Regulation: developments and directions

The European Medicines Agency (EMEA) is leading the way in establishing regulatory guidelines for biosimilar products. Its American counterpart, the Food and Drug Administration

(FDA), has yet to establish its own set of rules. The EMEA guidelines on biosimilar products have approached biosimilars as totally different entities from their originator products and highlighted the importance of the manufacturing process to biopharmaceuticals [28, 29]. In October 2005 the EMEA publicly acknowledged the complexity of biopharmaceuticals when they released an overarching document stating that biosimilars are not generics and should be subject to stringent testing before obtaining marketing authorisation [30]. In practice, these guidelines will translate into increased development costs associated with Phase III testing requirements and will likely affect the pricing of the final products, potentially resulting in less cost savings for biosimilars than for synthetic generic drugs.

The complexity of biopharmaceutical products is further reflected in the EMEA's release of concept papers specific to each class of biopharmaceutical molecules, such as those for recombinant human EPO products [31, 32]. As each family of biopharmaceutical molecules is unique, class-specific comparability guidelines were implemented. The concept papers specified the necessity of crossover studies in healthy volunteers and two adequately powered, randomised, double blind parallel clinical trials, to be performed separately for intravenous or subcutaneous delivery in the case of erythropoietin-like molecules. In addition to these studies, a 12-month immunogenicity comparison and a full pharmacovigilance plan are required. The choice of reference product is another important point brought up by the EMEA guidelines. Clear scientific justification should support the choice of the reference product used for the marketing authorisation application dossier and comparison should be performed in a clinical setting. Comparisons between routes of administration and other parameters of efficacy should also be scientifically assessed in the clinic.

However, there are still many areas that lack clear definition in the biosimilar development guidelines. For example, the EMEA's decision to approve a biosimilar product depends to a certain extent on the developers' ability to convince them that suitable pharmacovigilance plans will be implemented; however, the criteria of an acceptable pharmacovigilance plan remain to be determined. Keeping in mind the fact that immunogenicity generally appears over a long time frame in a minority of patients, a long-term pharmacovigilance plan with an adequate number of patients is essential for establishing the safety of biosimilars from an immunogenic point of view. The role of the physician is of particular importance as they are able to immediately report loss of efficacy or signs of immunogenicity. Another area that lacks proper definition is that non-clinical and clinical testing requirements for marketing authorisation may be reduced if the results from comparison of a biosimilar with the originator product are obtained through the use of ''sufficiently sensitive analytical systems''. The criteria for such analytical tools remain to be determined; even so, care must be taken when interpreting the results as even the most sophisticated analyses cannot substitute for properly conducted clinical trials. The consequence for the patient is that they may be treated with a drug that has not undergone the rigorous testing of a complete pre-marketing programme.

5 Conclusions

The development of biosimilars is clearly more complex than that of synthetic generic drugs, making it impossible to produce an exact copy of the originator protein. Slight differences in the product (including formulation and packaging) can have serious consequences for the patient. With the potential to reduce health care costs, it is clear that biosimilars are going ahead. However, patient safety should be of prime consideration, and ideally should prevail over financial considerations. Much work still needs to be done in order to prove that biosimilars are as safe and effective as their originator products.

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