

BIOPHARMACEUTICAL MOLECULES ARE NOT CREATED EQUALLY

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The number of biological products is increasing. Both new biopharmaceuticals for new indications as well as variants of existing molecules will be marketed soon. In addition, patents of a number of the first generation biopharmaceuticals will expire in the coming years, potentially opening the market for "biogenerics". For treating many diseases, such as chronic hepatitis C virus, chronic hepatitis B virus and multiple sclerosis, a number of alternative biological products could be available, which at face value may seem to be similar to the long established original product. Physicians confronted with many different alternatives have to be able to choose the best product for their patients to start treatment, and if and how to change to another product if a patient does not respond. In this article the authors consider the comparability of biopharmaceutical products

For most traditional pharmaceuticals many alternatives exist which are interchangeable. When a chemically synthesised drug comes off-patent competitors may seek marketing approval for their version of these products by providing evidence for comparability. To obtain the status of "generic product" the new manufacturer only has to show chemical identity and comparable pharmacokinetic behaviour in volunteers. The long-term experience with chemical drugs and the availability of sophisticated methods for analysis ensure that these generic products are safe and effective.

However, biological products differ in important aspects from traditional, chemically synthesised, drugs. The most obvious differences are molecular size, three dimensional shape, and process specific impurities. The size of a biopharmaceutical protein drug can approach 150,000 daltons, which is roughly 1,000 times the size of a standard drug molecule.¹

Whereas classical drugs are partly or completely chemically synthesised, biopharmaceuticals are produced in prokaryotic (no distinct nucleus) or eukaryotic (distinct nucleus) cell systems. The products have to be isolated from the cells or media with gentle purification and concentration methods, generally involving several steps. The final material has to be formulated with complex mixtures of excipients to ensure stability. In many cases the final material is freeze dried to avoid loss of activity during storage.²

All methods and processes of this complicated production and purification can greatly influence the nature and quality of the product. In contrast to synthesised drugs, where chemical tests can be used to detect predictable degradation products of the drug as well as impurities carried from the synthetic process, biopharmaceuticals have the potential to carry a large number of impurities from the cells and fermentation media in which they are produced. Since the molecules are large, there are a great number of potential degradation products of the biological entity likely to be present.

In biopharmaceuticals the coding sequence of the gene which is expressed is of great importance. Sometimes changes are introduced in the naturally occurring gene,

eg, the addition of methionine to enable expression in *Escherichia coli* or the removal of a cysteine to enhance stability. Other amino acid changes can be the result of mutations that occurred in the cell line from which the gene was cloned. These amino acids may or may not influence biological activity, and/or safety and antigenicity.

The host cell also influences the nature of the product and its properties. Production in prokaryotics lacks post-translational modification such as glycosylation. The lack of sugar residues may influence stability or biological characteristics such as pharmacokinetic behaviour and receptor binding.

Production in biological systems also leads inevitably to product heterogeneity. Mutation in the gene which is expressed may occur spontaneously. The fidelity of protein synthesis in bacterial hosts may be less than in mammalian systems. For instance in the case of interleukin-2 produced in *E coli*, methionine is replaced by norleucine in about 20 per cent of the molecules. This may be related to an imbalance in available amino acids by the forced expression of a product which may constitute 40 per cent of the total protein synthesis of the host cells.

The degree and type of glycosylation of the protein may also be variable due to the speed of production and the constituents of the production media. A number of other modifications may occur such as removal of N-terminal amino acids, abnormal folding by the formation of variant disulphide linkages and de-sialation of sugar residues. Also a number of degradations may occur such as oxygenation, deamidation and aggregate formation.³

To control the problem of product heterogeneity manufacturers must demonstrate that they can control the production process and are capable of producing batches that meet set specifications in a reproducible

way. Validated analytical methods and in-house standards are necessary to show batch to batch consistency.

The quality and safety of a biopharmaceutical is therefore highly dependent on the production process and experience. This is reflected in the different regulations and guidelines concerning the manufacturing process of biopharmaceuticals. If a company changes a single step in the production process, it needs to document that the safety and efficacy of the product is unchanged. Because the current analytical methods, including bioassays, are not capable of predicting biological behaviour, more often than not clinical trials are essential after modifications in the production process.

IMMUNOGENICITY

Immunogenicity is a sensitive parameter of the biological characteristics of a protein, because of the specificity of the immune system, which is capable of detecting small changes in three-dimensional structure.

The immunogenicity of biopharmaceuticals in patients illustrates how difficult comparisons of biopharmaceuticals can be. It shows that the current analytical methods are substantially insufficient to predict biological characteristics, making clinical comparison necessary. Because the immunogenicity of products can have serious consequences, it also shows the importance of full characterisation of a new product to ensure its safe use.

Several biopharmaceuticals have been reported to be immunogenic in patients including hormones, coagulation factors, cytokines, enzymes, monoclonal antibodies, fusion proteins, pegylated proteins and thrombolytic agents. This list shows that even so called "unmodified" proteins that are considered copies of natural products elicit antibodies in patients. This is unexpected because the patients should show immunotolerance.

The immunogenicity of human interferon alpha-2 (Hu IFN-alpha-2) products has been studied most extensively. The most striking feature of the immunogenicity of these products is the high variation in incidence reported. It ranges from 0 to more than 60 per cent.⁴

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Factors which contribute to this variation include:

- 1 Route, dose, frequency and duration of administration
- 1 Assays
- 1 Type of IFN
- 1 Type of disease
- 1 Genetic background
- 1 Unknown factors

It is obvious that route of administration, length of treatment and the total amount of biopharmaceutical administered influence the incidence of antibody formation. The most important reason for variation is the assay systems. These assay systems are either immunoassays, which identify binding antibodies, or bioassays, which identify neutralising antibodies. Because international standards may be lacking and material and methods differ between laboratories, it is impossible to compare results. There is a World Health Organization recommended way to express the neutralising activity of sera.⁵ However blind panel testing has shown that, even between experienced laboratories employing these calculation methods, results can differ more than 200-fold.⁶ So results on relative immunogenicity of different biopharmaceuticals should be interpreted with caution and are only valid if the preparations are compared in the same trials and the sera analysed in a single laboratory.

The type of product also influences immunogenicity. There have been many reports that IFN alpha-2a is more antigenic than Hu IFN alpha-2b.^{4,7} These interferons differ in a single amino acid. Moreover the gene for IFN alpha-2a is not present in the population, opening the possibility that this type of interferon carries a neo-antigen. However extensive studies have shown that the structural difference between the two products may not be the reason for the differ-

TABLE 1: PERCENTAGE OF PATIENTS PRODUCING NEUTRALISING ANTIBODY TITRES OF >19, 0-24 MONTHS AFTER START OF TREATMENT WITH TWO DIFFERENT BATCHES OF HUMAN INTERFERON BETA 1A

Months of treatment	BG 9015	Avonex
3	0	0
6	0	0
9	1	0
12	14	4
18	21	5
24	22	5

TABLE 2: PRODUCTS REPORTED TO INDUCE CLINICALLY RELEVANT ANTIBODIES

Product	Biological effects of antibodies induced
Growth hormone	Decrease in activity
Insulin	Resistance
Erythropoietin	Red cell aplasia
Factor VIII	Decrease in activity
Interferon alpha	Decrease in activity
Interferon beta	Decrease in activity
CD3 Mab	Increase CD3

ence in immunogenicity.⁸⁻¹⁰ After analysing the cause of the differences in immunogenicity between different batches of IFN-alpha-2a, its manufacturer concluded that oxygenation and aggregate formation, after prolonged storage at room temperature, of a human serum albumin (HSA) containing freeze-dried preparation was the reason for enhanced immunogenicity. Changing to a liquid, HSA-free formulation, and storage at 4°C might have reduced this problem.¹⁰

Sometimes the cause of immunogenicity is not so clear. A manufacturer of IFN beta-1a produced in chinese hamster ovary cells showed a significant reduction in

immunogenicity when the production site was changed (Table 1). BG 9015 was a batch used in a clinical trials and Avonex the marketed product. The only difference between the two batches was the production site. Extensive analysis of the two batches did not show any significant differences in physicochemical characteristics which influence antigenicity.^{11,12}

The consequences of immunogenicity can be severe.¹³ In Table 2 the biological effects of antibodies raised by the biopharmaceuticals are described. In some cases the efficacy of the product is lost. In the case of megakaryocyte derived growth factor and erythropoietin the antibodies were reported to interfere with the regulating activity of the natural counterparts leading to severe, and potentially lethal, thrombocytopenia and anaemia.

So the immunogenicity of biopharmaceuticals shows that the biological characteristics of a biopharmaceutical are unpredictable from its physicochemical or biological characterisation *in vitro*. In addition, it shows that while structural differences may not always lead to differences in biological behaviour, it simply cannot be assumed that "identical" structural properties means identical clinical behaviour. Moreover there may well be serious safety issues which can only be ruled out by comparative clinical trials. Because the immunogenicity sometimes only becomes apparent after prolonged treatment periods, clinical trials of long duration are necessary.

Finally, it needs to be born in mind that many of the currently available biopharmaceuticals provide life saving therapy for life threatening diseases. It is incumbent upon prescribing physicians and regulators to demand that substitute generic biopharmaceuticals have comparable safety and efficacy to the established original product and that this be compellingly demonstrated in randomised controlled trials.

REFERENCES

1. Bausch JN. Monoclonal antibodies. In: Stein S, editor. Fundamentals of protein biotechnology. New York: Marcel Dekker;1990.
2. Franks F. Storage stabilization of proteins. In: Franks F, editor. Protein biotechnology. New Jersey: The Humana Press; 1993.
3. Kumarasamy R, Bausch J, Kopcha D, Patel S, McGonigle E. An enzyme-linked immunosorbant assay (ELISA) for quantitation of adducts of granulocyte-macrophage colony stimulating factor (GM-CSF) and human serum albumin (HSA) in stressed solution mixtures. *Pharm Res* 1994;11:365-71.
4. Antonelli G. In vivo development of antibodies to interferons: An update to 1996. *J Interferon Cytokine Res* 1997;17:S39-47.
5. Grossberg SE, Kawade Y. The expression of potency of neutralizing antibodies for interferons and other cytokines. *Biotherapy* 1997; 10-1:93-98.
6. Schellekens H, Ryff J-C, Van Der Meide PH. Assays for antibodies to human interferon-alpha: The need for standardisation. *J Interferon Cytokine Res* 1997;17:S5-9.
7. Antonelli G, Currenti M, Turriziani O, Dianzani F. Neutralising antibodies to interferon alpha: relative frequency in patients treated with different interferon preparations. *J Infect Dis* 1991;163:882-5.
8. Hochuli E. Interferon immunogenicity: Technical evaluation of interferon alpha-2a. *J Interferon Cytokine Res* 1997;17:S15-23.
9. Palleroni AV, Aglione A, Labow M, Brunda MJ, Pestka S, Sinigaglia F, et al. Interferon immunogenicity: Preclinical evaluation of interferon alpha-2a. *J Interferon Cytokine Res* 1997;17:S23-29.
10. Ryff J-C. Clinical investigation of immunogenicity of interferon alpha-2a. *J Interferon Cytokine Res* 1997;17:S29-35.
11. Jacobs LD, Cookfair DL, Rudick RA, Herndon RM, Richert JR, Salazar A et al. Intramuscular interferon beta-1a for disease progression in relapsing multiple sclerosis. The Multiple Sclerosis Collaborative Research Group (MSCRG). *Ann Neurol* 1996;39:285-94.
12. Herndon RM, Jacobs LD, Coats ME, Goodkin DE, Mass MK, Richert JR et al. Results of an ongoing, open-label, safety-extension study of interferon beta-1a (Avonex) treatment in multiple sclerosis. *Int J MS Care* 1999. Available at www.ms-care.com/69912/page_02.htm (accessed 21 February 2002).
13. Ryff J-C, Schellekens H. Immunogenicity of rDNA derived pharmaceuticals: guidelines. *Trends Pharmacol Sci* 2002. In press.