

Biotech drugs: biological therapeutic agents

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The recent years has seen significant growth in a new therapeutic approach to the management of disease. Biological therapeutic agents, constitute a broad category of drugs, usually generated by recombinant techniques from living organisms. These therapies revolutionise the traditional approaches to drug design and development, and regulatory agencies have been swift in developing the necessary structures to ensure their optimal use.

Diverse disease processes are today being directly targeted by biological therapeutics, and these agents are also being used as conjugates to conventional small drug molecules, in order to direct the drug towards specific body compartments. This article reviews the general aspects that surround the types and modes of action of these agents, and highlights issues that contribute towards development of resistance to biologic drugs.

Introduction

For several years, science has been credited with the continual development of

new medicinal products to treat or manage a diverse range of medical conditions. Pharmaceutical companies have stroven to produce drugs with higher efficacies and improved safety profiles, while enhancing the therapeutic repertoire with agents capable of addressing hitherto unmet needs.

Traditionally, such drugs have largely consisted of low molecular weight molecules which were often the result of chemical engineering of a naturally-found product. Years of research have generated methodologies by which such chemical entities can be synthesized, structurally characterized and analytically quantified,

leading for example, to the establishment of defined protocols for shelf-life stability predictions, pharmacokinetic calculations and bio-equivalence studies.

The recent years, have seen the emergence of a totally new class of therapeutic agents, which are the result of large strides being accomplished in the biotechnology arena. Biological drugs (or simply biologics), as these entities are known, are usually based on endogenous biocompounds and consist of high molecular weight protein structures or short peptides. Rather than being chemically synthesized, biological drugs are usually produced by recombinant technologies and gene expression methodologies, i.e. they are produced in laboratories by a living system, and are the product of biosynthetic pathways.

The Food and Drug Administration (FDA) adopts the US Public Health Service Act (Section 351) definition which states that a biological product is a "virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, or analogous product, ... applicable to the prevention, treatment, or cure of a disease or condition of human beings."¹ The European Medicines Agency (EMA) runs its own Biologics Working Party (BWP) which operates under the Committee for Medicinal Products for Human use (CHMP). The main role of the BWP is to provide recommendations on all matters relating directly or indirectly to quality aspects and safety in relation to quality of biological and biotechnological medicinal products.² More recently, the CHMP has also established a Biosimilar Medicinal Products Working Party (BMWP) with the mandate of providing recommendations on the non-clinical and clinical matters relating directly or indirectly to similar biological medicinal products.³ This need has arisen from the fact that unlike conventional small molecule drugs, biologics produced by generic drug companies (biosimilars) may have similar ingredients and possess similar activities to the authorized product but may be unidentical due to differences in production methodologies, gene expression techniques etc. The complex 3D structure and highly specific physiological, chemical and physical properties of these products makes it a major challenge for generic producers to provide evidence for the biosimilarity of their product to that of the originator.⁴

Actions of biological drugs

The production of biomolecules has been extensively developed to deal with dysfunctional protein counterparts, deficient systemic production of growth factors, deregulated cytokine activity and molecular targeting. The high throughput technology available today has been instrumental in the identification of molecular mechanisms of disease with the potential to specifically target key molecules related to the condition. Table 1 shows a non-exhaustive list of biotech drugs that are currently approved by the Food and Drug Administration (FDA) for targeted therapy.

The production of insulin is the pioneering work in biotechnology. Stanley Cohen and Herbert Boyer produced insulin in 1973 for the treatment of diabetes. The human gene of insulin was isolated and inserted in bacteria for large scale protein synthesis.⁵ This was the start of the use of recombinant technology for the production of pharmaceuticals. Another success story in the biotechnology field is the production of erythropoietin (Epo). Epo is a cytokine that stimulates erythrocyte production in response to a low oxygen tension.⁶ The production of recombinant human erythropoietin (rhEpo) to treat anaemias associated with chronic renal failure has proven to be highly effective and safe, giving a remarkable therapeutic window.^{7,8} The main concerns associated with the long-term administration of

erythropoietin are the frequent need for parenteral administration, the development of anti-Epo antibodies and the cost of the treatment. Improved erythropoietic stimulating agents were developed through chemical modifications that enhance biological activity. The hyperglycosylated rhEPO analogue, darbepoetin alfa contains five N-linked carbohydrate chains resulting in increased molecular weight and greater negative charge. The serum half life is 3 fold longer than rhEpo and due to the increased in vivo potency, the biomolecule can be administered less frequently.^{9,10} In addition, the Epo mimetic peptide, Hematide™, binds to the erythropoietin receptor and elicits signal transduction giving normalised haemoglobin levels for a period of at least one month following one intravenous administration.¹¹ Hematide™, currently at an advanced clinical trial stage, may offer enhanced compliance and an improved risk-benefit ratio.

Hyperglycosylation is only one example of chemical modifications that have been developed for enhanced therapeutic window of biotherapeutics. Granulocyte Colony Stimulating Factor (G-CSF) is another member of the cytokine family that includes Epo. G-CSF has been engineered and produced as a biotech drug for the treatment of neutropenia and its use has increased substantially due to drug-induced neutropenia, associated with administration of the chemotherapeutic agents. Increased

serum retention and stability of recombinant G-CSF has been achieved by PEGylation, the covalent conjugation with polyethylene glycol (PEG) moiety.¹²

In addition to systemic therapeutic use of factors and cytokines, biomolecules that target specific cells and the cell environment are gaining importance. These mainly consist of antibodies with various molecular functions.

Antibody-dependent cellular cytotoxicity

Unconjugated monoclonal antibodies are produced to target specific antigens/receptors to elicitate an immune response. The recombinant, humanized monoclonal antibody trastuzumab specifically targets cells expressing the human epidermal growth factor receptor 2 (HER2).¹³ Following binding an antibody-dependent cell-mediated cytotoxicity is induced. HER2 is overexpressed in many adenocarcinomas, and promising results are being reported in breast adenocarcinomas.¹⁴ It is important to classify patients as HER2-positive to ensure beneficial responses. In B-cell non-Hodgkin's Lymphoma the surface marker CD20 is targeted (Rituximab) initiating an antibody-dependent and complement-dependent cell mediated cytotoxicity.¹⁵

Inhibition of ligand binding

A human monoclonal antibody targeting epidermal growth factor receptor (EGFR), panitumumab, binds the receptor with high affinity, blocking receptor-mediated signaling by competing out the natural ligand. Panitumumab inhibits the growth and survival of tumour cells expressing EGFR¹⁶ and is FDA approved for the treatment of metastatic colorectal cancer. Antibodies can also bind to ligands and prevent ligand-receptor binding. Infliximab binds and blocks the action of tumour necrosis factor alpha (TNF α). Infliximab is a chimeric monoclonal antibody developed in mice and subsequently modified into a human (humanized) antibody.

Omalizumab is a recombinant DNA-derived humanized IgG1 monoclonal antibody that selectively binds to human immunoglobulin E (IgE), thus inhibiting the binding of IgE to high affinity receptors on the surfaces of dendritic cells, mast cells and basophils. It is indicated for the management of asthma, as it directly reduces the activity of IgE on the activation of these pro-inflammatory cells. Moreover, de novo generated IgE-receptor carrying cells, are

Table 1. Therapeutic biomolecules

Name	Type	Molecular target	Condition
Insulin	Systemic Factor	Glucose metabolism	Diabetes
Erythropoietin	Systemic Factor	Erythropoiesis	Renal failure
G-CSF	Systemic Factor	Neutrophils	Neutropenia
Trastuzumab	Humanized monoclonal antibody	Human epidermal growth factor receptor 2 (Her2)	Breast adenocarcinomas
Panitumumab	Humanized monoclonal antibody	Epidermal growth factor receptor (EGFR)	Colorectal carcinoma
Gemtuzumab ozogamicin	Conjugated humanized monoclonal antibody	CD33	Acute myeloid leukemia
Rituximab	Chimeric	CD20	B cell non-Hodgkin's lymphoma
Omalizumab	Humanized monoclonal antibody	IgE	Asthma
Infliximab	Chimeric monoclonal antibody	TNF	Rheumatoid arthritis; Crohn's disease

exposed to lower circulating concentrations of free IgE, and this has been shown to result in a decreased expression of surface IgE receptors on these cells. The drug is administered sub-cutaneously in repeated doses, to patients who satisfy specific diagnostic criteria, and clinical studies have shown this to result in a significant reduction of exacerbations of asthma.

Conjugated antibodies

Genetically engineered antibodies or antibody fragments have the capacity to target tumour cells or the tumour environment and facilitate a response by directing a chemotherapeutic drug or by recruiting the immune system, as evidenced in pre-clinical studies. The conjugation of a cytotoxic agent to a recombinant antibody results in the specific binding of the antibody to targeted cells, followed by internalization and the delivery of the agent. The chemotherapeutic drug, Gemtuzumab ozogamicin consists of a recombinant, humanized anti-CD33 monoclonal antibody attached to the cytotoxic agent calicheamicin. The antibody targets CD33-positive leukemic blasts, followed by delivery of calicheamicin which binds to the minor groove of DNA causing a double strand break.¹⁷

Immunocytokines are fusion proteins that recruit the immune system to tumour cells as is the case of IL2 fusions with EpCAM and L19. The fusion protein (tucotuzumab celmoleukin) concentrates the active interleukin-2 (IL-2) molecule at tumour tissue expressing the cell adhesion molecule, EpCAM.¹⁸ The L19-IL2 is a fusion protein composed of an antibody fragment against a fibronectin isoform present in neovascularisation.¹⁹ This allows targeting of angiogenesis. In all these cases the localized IL-2 molecule stimulates a cytotoxic T-cell antitumour immune response. Specific targeting of cells through conjugated antibody technology is envisaged to emerge as a major biological therapeutic approach.

Resistance to biological therapeutic agents

Regular use of conventional drugs is well known to often lead to gradual development of tolerance, and the mechanisms of this often involve events which occur at the receptor level. A distribution of observed efficacies in a given population is also often evident, with a proportion of individuals

sometimes being clinically unresponsive to a specific therapeutic agent. Biological therapies may also exhibit similar effects, though the mechanisms are usually more complex.

Protein expression levels

The expression levels of various endogenous proteins may be altered in disease, and may influence the efficacy of therapeutic biological agents. Overexpression of the membrane-associated glycoprotein mucin-4 (MUC4) for example, can mask the HER2 receptor, and prevent binding of trastuzumab. Cell culture-based experiments have shown that increased signalling from HER2/HER3 and HER2/EGFR complexes in HER2+ve cells, contribute to development of resistance to trastuzumab.²⁰

The situation with interferon- α (IFN α), used therapeutically to target several neoplasms such as chronic myeloid leukaemia (CML), is more complex. In vitro work on IFN α -resistant cell lines, has shown these cells to show altered expression of 39 different genes, which are involved in such diverse functions as signal transduction, apoptosis, transcriptional regulation and cell growth.²¹

Besides their direct role in the transfer of various drug molecules across cell membranes, drug transporters, such as P-glycoprotein (P-gp), may also influence biological therapeutic agents through indirect mechanisms. Tumour Necrosis Factor-alpha (TNF α)-based therapies are used to induce cell-death (apoptosis) in anti-cancer therapeutics.

In order for these therapies to exert their apoptotic function, another family of proteins called caspases must come into play. P-gp, which is occasionally overexpressed in some leukaemias and solid tumours, has been recognised to inhibit caspase-3 activation, and therefore downstream apoptotic pathways, causing an effect that is clinically observed as a resistance to TNF α -based therapies.²²

Genetic variability

Resistance to protein or peptide-based therapies may also arise from the presence of DNA variability in specific genes, and several examples of this exist in literature. Of interest is the association of oncogenic mutations in the phosphoinositide 3-kinase (PI3K) pathway and trastuzumab resistance.²³ This is in line with the observations that trastuzumab action involves the

inactivation of the PI3K pathway.²⁴ Hence characterisation of the activity of the PI3K pathway is a potential predictive biomarker panel for resistance associated with trastuzumab use.

Immunogenicity

All biological therapeutic agents are potentially immunogenic, and antibodies to these agents may be detectable in some patients. For example about 6% of rheumatoid arthritis patients on adalimumab have detectable levels of antibodies to the drug, while in the case of infliximab, antibodies are detected in about 11% of RA patients. Indeed, this was proposed as an explanation for the observed progressive decrease in infliximab efficacy over time, which is not clinically observed with adalimumab.²⁵

Omalizumab, a recombinant humanized monoclonal antibody to immunoglobulin E (IgE) has been recently licensed for anti-asthma therapy. Rare hypersensitivity reactions have been reported following use of this drug, and these include bronchospasm, hypotension, syncope, urticaria, and angioedema of the throat or tongue. Such reactions have been reported to occur as early as after the first dose of omalizumab, but also beyond 1 year after beginning regularly administered treatment.^{26,27}

Other mechanisms

It is often difficult to suitably explain the causes of drug resistance. For example, studies have shown that the efficacy of infliximab appears to decrease over time with continuous usage, and this clinical observation is evident after 6 months of treatment.

However, this has not been reported to occur with adalimumab – another TNF α antibody, or etanercept - a TNF α inhibitor that mimics the actions of endogenous soluble TNF α receptors. It has been suggested that lymphotoxin- α (LT α), a member of the TNF β family, which has been identified in the synovial fluid of infliximab-resistant RA patients, may have a role in the development of this resistance, but the exact mechanism remains to be elucidated.²⁸

Conclusion

The recent years have seen a rapid growth in the development of new biological drugs, as therapeutic modalities that offer improved outcomes over conventional

medicines. These new components of our therapeutic arsenal, mandate healthcare professionals to update the way they look at drugs, and opens the horizon to new potentials for disease targets and mechanisms of drug action. Their relatively high cost compared to conventional small molecule drugs remains to be addressed. Of particular relevance is the low accessibility of these drugs by resource-poor countries (RPCs).

The editorial of the Journal of Rheumatology has called for a different pricing strategy for biologics to be applied in RPCs, coupled to the provision of support for the use of these therapies.²⁹ The economics of using more expensive medication should be assessed together with the projected

Practice points

- Biological agents offer novel approaches to disease management, due to their ability to interfere with specific processes which cannot be targeted by conventional molecules.
- These drugs may be designed to emulate endogenous biocompounds, inhibit specific proteins, recruit endogenous molecules to specific tissues or target conventional agents to their sites of action.
- Similar to conventional therapies, resistance to biological therapeutic agents may also occur, though the mechanisms may often be different.
- Though the direct cost of these therapies is higher than conventional agents, their optimal economic use should include an assessment of improved efficacies, decreased long term medical care costs, improved quality of life and increased economic productivity.

decreased medical and health care personnel costs and increased productivity due to a lowered use of sick-leave, improved quality of life, and less early retirement events by

patients. Moreover, biologics may often be used in combination with conventional drugs, in order to optimize the respective cost-benefit ratios.

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