Delivery of biopharmaceuticals
Introduction

• Biopharmaceuticals, also known as biologicals or biologics, are medicines in which the active is derived from a biological (usually non-plant) source.

• Biopharmaceuticals include vaccines, proteins and peptides, nucleic acids and carbohydrates, all chemical components that exist in nature. Also, cell types such as the emerging area of transplanted stem cells and engineered tissues.
<table>
<thead>
<tr>
<th>Biopharmaceutical class</th>
<th>Example</th>
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<td>Peptides</td>
<td>Oxytocin</td>
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<td>Proteins</td>
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<td>Enzymes</td>
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<td>Monoclonal antibodies</td>
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<td>Cytokines</td>
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<td>Hormones</td>
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<td>Hematopoietic growth factors</td>
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<td>Vaccines</td>
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<td>Nucleic acid drugs</td>
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<td>Oligonucleotides</td>
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<td>Cell-based therapies</td>
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<td>Carbohydrates</td>
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<td>Low molecular weight heparin</td>
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Introduction

➢ Protein and peptide drugs
  • Production
  • Delivery issues
  • Delivery systems

➢ Vaccines
  • Production
  • Delivery issues
  • Delivery systems

➢ Nucleic acid drugs
  • Production
  • Delivery issues
  • Delivery systems
Protein and peptide drugs

- Proteins are composed of 20 known individual amino acids linked by amide bonds.
- Peptides differ from proteins mainly in the number of amino acids contained within each molecule. Peptides are generally defined as having less than 50 amino acids, while proteins usually contain hundreds of amino acids and have a tertiary (folded) structure. However, insulin with 51 amino acids is defined as a peptide.
- Endogenous proteins are synthesized in the cell in an amino acid sequence that is defined by a specific nucleotide base pair sequence.
Protein and peptide drugs

• There is post translational modification, including glycosylation and protein folding to give the functional three-dimensional structure. Endogenous bioactive peptides are also synthesized within the cell and are normally the result of cleavage of larger proteins to give the peptide active.

• The peptide therapeutic classes mostly comprise peptide hormones, such as insulin and calcitonin as well as endogenous peptide analogues, such as goserelin.
Production (Protein and peptide drugs)

• Protein drugs are produced in
  1. Mammalian cells: e.g. the Chinese Hamster Ovary (CHO) cell line.
  2. Bacteria: e.g. Escherichia coli (E. coli).
  3. Yeast cells.
  4. Transgenic animals (production of human antithrombin in goats’ milk)
  5. Plant sources (trastuzumab in the Nicotiana benthamiana specie using viral gene expression systems)

The gene of interest is transfected into the cells and the cells are grown in a bioreactor. The protein product is isolated by cell lysis and centrifugation/filtration and the protein is purified using chromatographic techniques.
**Fig. 46.2** • The production of biopharmaceuticals.
Production processes are normally patented and as a consequence, it is impossible, once the protecting patents have expired, for other companies to produce exactly the same therapeutic protein with identical glycosylation patterns without access to the original bioreactor procedures.

This led to a new category of medicine made by a separate manufacturer after patent expiry, which are known as ‘biosimilars’ or ‘biobetters’.

Biosimilars must be comparable with respect to a number of key indicators in order to be considered biosimilars of reference marketed products. A number of biosimilars have been introduced and the biosimilars currently available in Europe are listed in Table 46.3.
<table>
<thead>
<tr>
<th>Therapeutic</th>
<th>Biosimilar</th>
<th>Biosimilar manufacturer</th>
<th>Reference product</th>
<th>Reference product manufacturer</th>
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<tbody>
<tr>
<td>Human Growth Hormone</td>
<td>Omnitrope</td>
<td>Sandoz</td>
<td>Genotropin</td>
<td>Pfizer</td>
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<td>Valtropin</td>
<td>Biopartners</td>
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<td>Absamead</td>
<td>Medice Arzneimittal</td>
<td>Eprex</td>
<td>Janssen-Cilag</td>
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<td>Pitter</td>
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<td>Binocrit</td>
<td>Hospira</td>
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<td>Epoetin alpha Hexal</td>
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<td>Silapo</td>
<td>Hexal Biotech</td>
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<td>STADA Arzneimittal</td>
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<td>Granulocyte Colony Stimulating Factor</td>
<td>Filagrasim</td>
<td>Hexal Biotech</td>
<td>Neupogen</td>
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<td>Tevagrastim</td>
<td>Teva Pharma</td>
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(Adapted from Drutitsaris et al, 2011.)
Delivery issues (Protein and peptide drugs)

• The delivery issues surrounding protein and peptide drugs are divided into two main categories:
  a) Maintaining stability on storage
  b) The optimization of in vivo efficacy.

Storage stability issues may be classified according to the chemical and physical origins of protein instability.
Delivery issues (Protein and peptide drugs)

➢ Chemical instability:
  • Deamination (base catalysed hydrolysis of asparagine to aspartic acid)
  • Peptide bond hydrolysis
  • Oxidation of proteins

➢ Physical instabilities:
  • Denaturation (alter their native secondary or tertiary structure) on exposure to heat, extremes of pH or organic solvents.
  • Aggregation in solution (hydrophobic attraction or covalent bonds)
Delivery issues (Protein and peptide drugs)

• The optimization of in vivo efficacy:
  ➢ Difficulty in crossing biological, lipid rich membranes (e.g. GIT and brain capillary endothelial cells) because of hydrophilic character, size, degradation figure.
  ➢ Inactivation if taken orally (only 2 oral peptide drugs on the market peptides ciclosporin and desmopressin- both cyclic peptides).
  ➢ Rapid clearance for proteins injected intravenously
  ➢ Immunogenicity, where proteins generate neutralising antibodies, and drug inactivation.
Fig. 46.4 - The poor transport of proteins and peptides across biological membranes.
Delivery systems (Protein and peptide drugs)

➢ Protein stabilization:

• The formulation of proteins and peptides involves preventing chemical degradation and enhancing in vivo activity

• To prevent chemical degradation:

  ✓ A low pH is preferred (pH = 3–6) as this limits the reactivity of nucleophiles. Nucleophilic attack leads to deamination or amine terminal cyclization.

  ✓ Drying, especially freeze-drying may be used to stabilize proteins against a variety of degradative influences (e.g. hydrolytic peptide bond cleavage)

  ✓ To prevent oxidative damage, by reducing the head space in the final vial. Or adding metal chelating agents, e.g. (EDTA).

  ✓ Protein aggregation may also be prevented by the inclusion of various sugars, glycerol, arginine and urea that bind to hydrophobic regions.
Delivery systems (Protein and peptide drugs)

➢ Protein delivery:

• Proteins are administered: parenterally (intravenously, subcutaneously or intramuscularly). The nasal and pulmonary route, have also been attempted.

• Pharmaceutical proteins are generally rapidly cleared from the blood with short plasma half-lives. One method of prolonging the circulation time of proteins is by the use of (PEG ) conjugation.
Peptide delivery:

• Peptides, containing 2–50 amino acid residues are largely administered parenterally although there are a few marketed products in which peptides are administered via the nasal route (e.g. calcitonin nasal spray – 32 amino acids).

• For the past three decades, there has been a huge focus on alternative forms of insulin delivery (administered parenterally-subcutaneously). There have been hundreds of studies examining the feasibility of delivering insulin via the oral route, but at the present time there are no commercially available insulin oral dosage forms.
Vaccines

Vaccines consist of antigens which produce antibodies and induce immunological memory, enabling the immune system to recognize and destroy specific pathogens if exposed to the pathogenic molecules a second time.

There are three types of vaccines:

1. Live organisms, which have been attenuated to ensure that they do not cause disease;
2. Inactivated vaccines (inactivated by heat or chemical means);
3. Subunit vaccines.
Production (vaccine)

• Bacterial vaccines are grown in bioreactors
• Viral vaccines are produced in fertilized chicken eggs
• Once the viral or bacterial vaccine has been harvested it is inactivated using chemical methods or heat, prior to formulation.
• Vaccine recombinant subunits are grown in host cells, by inserting the gene for the antigen into bacterial, yeast or mammalian cells and growing multiple copies of the antigen.
• The antigen is isolated from the cells using ultracentrifugation and chromatographic means.
Delivery issues (vaccines)

• It is important to maintain vaccines structure to prevent antigen degradation and ensure potency (by storing them in cold conditions).
• It is also desirable to prevent unwanted bacterial growth and to ensure a sufficiently high and prolonged immune response as this will allow for fewer vaccination events.
• With subunit vaccines, it is necessary to present the antigenic proteins in particulate form to enable efficient uptake by antigen presenting cells.
Delivery systems (vaccines)

• Most multiple use vaccines contain a preservative.
• Vaccines may also contain antibiotics to prevent unwanted bacterial contamination.
• In order to circumvent the considerable expense associated with the maintenance of a cold chain storage conditions, researchers have prepared vaccine-sugar glasses in which a vaccine is mixed with trehalose and sucrose and dried on a membrane to be hydrated when required. (dry vaccine formulation)
Delivery systems (vaccines)

• Particulate delivery systems such as emulsions, liposomes and virosomes enable the subunit vaccines to be presented in a particle as it would be if it was still part of an infectious organism, allowing the antigen to then be taken up by antigen presenting cells.

• Intradermal vaccination using microneedles offers the advantages of painless delivery.

• Microneedles, which are 500–750 µm long, deliver their cargo into the epidermis or just below it and do not penetrate to the nerve endings, making the injection painless.

• Microneedles may be fabricated from solid, hollow or dissolvable materials and dissolvable microneedles have been fabricated from maltose and amylopectin.
Nucleic acid drugs

- Nucleic acid-based drugs fall into three classes:

1. Antisense oligonucleotides. single stranded chains of nucleotides which inhibit translation of viral messenger RNA.
2. Small interfering ribonucleic acids (siRNAs). inhibit translation by hydrolytic degradation of messenger RNA

Deoxyribonucleic acid (DNA) is the constituent material of genes and ribonucleic acid (RNA) is the constituent material of messenger and transfer RNA. These nucleic acids consist of double stranded chains of nucleotides.
Nucleic acid drugs

• A number of diseases may be traced to the mutation of various genes and thus have a genetic basis.

• Genes give rise to the cell’s proteins via transcription (messenger RNA synthesis) and translation – protein synthesis.
Nucleic acid drugs

• proteins are the functional components of the cell. Gene mutations will thus alter the resulting protein and such alterations may lead to disease.

• Gene therapy can be used to replace a mutated gene and thus achieve a functioning protein. Delivery vectors are required to achieve the gene therapy goal.

• Example of in humans is the licensed gene medicine, Gendicine. Gendicine contains wild type p53 to replace mutated p53 in cancer cells and is delivered in an adenoviral vector.
Production

• The two marketed gene therapeutics (Gendicine and Rexin G) are delivered using viral vectors. Gendicine, which is delivered in an adenovirus vector, is produced as a viral particle.

• Synthetic (chemical compound) vectors can be used for gene therapy, the gene product is delivered as a bacterial plasmid. Plasmids containing the gene of interest are grown in E. coli cell lines and purified by cell lysis, filtration, chromatographic separation and centrifugation.

• siRNA and oligonucleotides are synthesized chemically with many companies offering custom synthesis for particular siRNA or oligonucleotide sequences.
Delivery issues

• The main delivery issues surrounding nucleic acid drugs are:
  • a) poor plasma stability of DNA, siRNA and oligonucleotides
  • b) the inability of a large polar (negatively charged at physiological pH) molecule such as DNA, or even double stranded siRNA to cross the lipid rich plasma cell membrane.
• A further issue surrounding DNA delivery is the fact that therapeutic DNA must gain entry to the cell nucleus in order to produce its therapeutic product, the functional protein.
• Entry to the nucleus is limited largely to cell division events. These delivery issues mean that gene and siRNA therapeutics have an absolute requirement for a delivery system.
Delivery systems

• The delivery of genes in commercial gene therapies has so far been achieved using viruses.

• **Gendicine**, the world’s first gene therapeutic agent, and currently only licensed for use in China, comprises the wild type p53 gene within an E1 deleted gene adenovirus for the treatment of head and neck cancers and is administered intratumorally (not for metastatic).

• **Rexin G** is available in the Philippines for the treatment of metastatic lesions is : which is a replication incompetent retroviral (murine leukemia virus) particle, bearing the human Cyclin G 1 gene. The Cyclin G 1 gene causes apoptosis and necrosis in dividing tumour cells and in the cells of the tumour vasculature.
Delivery systems

• Synthetic gene vectors have been explored, using poly(propylenimine) dendrimers, liposomes and naked DNA. Good preclinical efficacy (tumour regression) data has been obtained with the poly(propylenimine) dendrimer gene therapy system.

• The delivery of siRNA has been accomplished clinically using a cyclodextrin based nanoparticle carrier covered with PEG. Gene silencing of the M2 subunit of ribonuclease reductase was achieved in human melanoma tissue in this pivotal human study.
Key points

• Biopharmaceuticals are medicines which contain active agents of biological origin and include: enzymes, monoclonal antibodies, cytokines, hematopoietic blood actors, peptides, genes, siRNA, oligonucleotides, vaccines and carbohydrates

• Biopharmaceuticals are usually commercially produced in mammalian cell bioreactors, purified by centrifugation and/or filtration and characterized using various spectroscopic, chromatography and calorimetric techniques.
Key points

• Biopharmaceuticals have specific formulation and delivery issues: a) they are easily degraded/inactivated on storage and b) they are easily cleared in vivo and thus have difficulty reaching their therapeutic target.

• Delivery solutions or this class of medicines involve the use of poly(ethylene glycol) conjugates to prolong the activity of proteins, polymer matrices to sustain the activity of peptides, the use of vaccine delivery systems and vaccine adjuvants to enhance the prophylactic immune response, and the use of viral and synthetic vectors to deliver gene therapies.