TARGETED DRUG DELIVERY SYSTEMS AND BIOTECHNOLOGICAL PRODUCTS: INTRODUCTION

Many diseases occur as a result of defects or errors in the genes involved in producing essential enzymes or proteins in the body. The genes are coded in *deoxyribonucleic acid* (DNA), helical double-stranded molecules folded into chromosomes in the nucleus of cells. The Human Genome Project was created several years ago to sequence the human genome. This national effort is now yielding information on the role of genetics in congenital defects, cancer, disorders involving the immune system, and other diseases that have a genetic link.

The emerging genetic basis of disease will provide novel opportunities for the development of new drugs to treat these disorders, particularly in the field of biotechnology. The discovery of recombinant DNA (rDNA) technology and its application to new drug development has revolutionized the biopharmaceutical industry. Previously, the pharmaceutical industry relied on the use of relatively simple small drug molecules to treat disease. Modern molecular techniques have changed the face of new drug development to include larger, more sophisticated and complex drug molecules. These large biopharmaceuticals have enormous potential to treat disease in novel ways previously unavailable to small drug molecules. As a result, *biotechnology,* or the use of biological materials to create a specific drug product, has become an important sector of the pharmaceutical industry and accounts for the fastest-growing class of new drugs in the market. Nucleic acid, protein and peptide drugs, and diagnostics are the main drug products emerging from the biopharmaceutical industry.

BIOTECHNOLOGY

Protein Drugs

The human genome produces thousands of gene products that prevent disease and maintain health. Many may have therapeutic applications if supplemented to normal or supraphysiologic levels in the body. Most of the biologic molecules listed in are normally present in the body in small concentrations but are used for certain therapeutic indications. For example, some diseases such as insulin-dependent diabetes result from insufficient production of a natural product, in this case insulin. For these patients, the treatment is to supplement the patient's own insulin production with recombinant human insulin (eg, Humulin). Similarly, human recombinant growth hormone (Protropin, Nutropin) and glucocerebrocidase (Ceredase, Cerezyme) are used to treat growth hormone deficiency and Gaucher's disease, respectively.

Table 18.1 A Sample of Approved Recombinant Drugs

Drug	Indication	Pharmacokinetics	Year Introduced, Company (Trade Name)
Aldesleukin; interleukin-2	Renal cell carcinoma	Half-life = 85 min; *Cl* = 268 mL/min	1992 Chiron (Proleukin)
Alteplase	Acute myocardial infarction	Half-life < 5 min; *Cl* = 380–570 mL/min; $V_d \approx$ plasma volume	1987 Genentech (Activase)
	Acute pulmonary embolism		1990 Genentech (Activase)
Antihemophilic factor	Hemophilia B		1992 Armour (Mononine)
Antihemophilic factor	Hemophilia A	Half-life = 13 hr	1992 Genetics Institute, Baxter Healthcare, Bayer (ReFacto, Recombinate, Kogenate, Helixate FS)
Agalsidase-beta; α-galactosidase A	Fabry's disease	Half-life = 45–102 min; nonlinear kinetics	2003 Genzyme (Fabrazyme)
Anakinara; IL-1 receptor antagonist	Rheumatoid arthritis	Half-life = 4–6 hr	2001 Amgen (Kineret)
β-Glucocerebrosidase;	Type I Gaucher's disease		1991 Genzyme (Ceredase)
Glucocerebrocidase	Type I Gaucher's disease		1994 Genzyme (Cerezyme)
CMV immune globulin	CMV prevention in kidney transplant		1990 Medimmune (CytoGam)
DNase	Cystic fibrosis		1993 Genentech (Pulmozyme)
Drotrecogin-α;	Severe sepsis	*Cl* = 40 L/hr	2001 Lilly (Xigris)

activated protein C			
Erythropoietin	Anemia associated with chronic renal failure	Half-life = 4–13 hr	1989 Amgen; Johnson & Johnson; Kirin (Epogen); 1990 Ortho Biotech (Procrit) 1990 Amgen; Ortho Biotech (Procrit) 1993 Amgen; Ortho Biotech (Procrit)
	Anemia associated with AIDS/AZT		
	Anemia associated with cancer and chemotherapy		
Factor VIII	Hemophilia A		1993 Genentech; Miles (Kogenate)
Filgrastim; G-CSF	Chemotherapy-induced neutropenia	Half-life = 3.5 hr; V_d = 150 mL/kg; *Cl* = 0.5–0.7 mL/kg/min	1991 Amgen (Neupogen)
	Bone marrow transplant		1994 Amgen (Neupogen)
Human insulin	Diabetes		1982 Eli Lilly, Genentech (Humulin)
Interferon-α-2a	Hairy cell leukemia;	Half-life = 5.1 hr; V_d = 0.4 L/kg; *Cl* = 2.9 mL/min/kg	1986 Hoffmann-La Roche (Roferon-A)
	AIDS-related Kaposi's sarcoma		1988 Hoffmann-La Roche (Roferon-A)
Interferon-α-2b	Hairy cell leukemia;	Half-life = 2–3 hr	1986 Schering-Plough; Biogen (Intron A)
	AIDS-related Kaposi's sarcoma		1991 Schering-Plough; Biogen (Intron A)
Interferon-α-n3	Genital warts		1989 Interferon Sciences (Alferon N injection)
Interferon-β 1b	Relapsing/remitting multiple sclerosis	Half-life = 8 min–4.3 hr; *Cl* = 9.4–28.9 mL/kg/min; V_d = 0.25 –2.9 L/kg	1993 Chiron; Berlex (Betaseron)
Interferon-β 1a	Multiple sclerosis	Half-life = 8.6–10 hr	1996 Biogen (Avonex); 2002 Serano (Rebif)
Interferon-γ-1b	Management of chronic granulomatous disease		1990 Genentech (Actimmune)
Human growth hormone	Short stature caused by human growth hormone deficiency		1994 Genentech (Nutropin)
Hepatitis B vaccine, MSD	Hepatitis B prevention		1986 Merck; Chiron (Recombivax HB) Smith Kline
			1989 Beecham; Biogen (Engerix-B)
Laronidase; α-L-iduronidase	Mucopolysaccharidosis I	Half-life = 1.5–3.6 hr; *Cl* = 1.7–2.7 mL/min/kg; V_d = 0.24–0.6 L/kg	2003 Biomarin (Aldurazyme)
Pegadamase (PEG-adenosin)	ADA-deficient SCID		1990 Enzon; Eastman Kodak (Adagen)
PEG-L-asparaginase	Refractory childhood acute lymphoblastic leukemia		1994 Enzon (Oncaspar)
Reteplase; plasminogen activator	Acute myocardial infarction	Half-life = 0.2–0.3 hr; *Cl* = 7.5–9.7 mL/min/kg	1996 Boehringer Mannheim (Retavase)
Sargramostim (GM-CSF)	Autologous bone marrow transplantation		1991 Hoechst-Roussel; Immunex (Prokine)
	Neutrophil recovery following bone marrow transplantation		1991 Immunex; Hoechst-Roussel (Leukine)
Somatropin	hGH deficiency in children		1987 Eli Lilly (Humatrope)

Somatrem			1985 Genentech (Protropin)
Tenecteplase	Acute myocardial infarction	Half-life = 90–130 min; *Cl* = 99–119 mL/min; V_d ≈ plasma vol.	2002 Genentech (TNKase)

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In contrast, *interferons* are proteins produced by the immune system in response to viral infection and other biologic inducers. When infection or cancer surpasses the capacity of the body's immune system, recombinant interferons (Roferon-A, Intron A, Alferon N, Actimmune, Infergen, Rebif) or other immune-enhancing molecules can be used to boost immunity. Recombinant interferons and interleukins (Proleukin, Neumega) are therefore used to strengthen the immune system during infection, immunosuppression, cancer, and multiple sclerosis. Erythropoetin and derivatives (Epogen, Procrit, Aronesp) and growth factors (Prokine, Leukine, Neupogen, Becaplermin) are also used to stimulate red and white cell production for anemia or immune suppression following chemotherapy. These molecules were originally available only by purification from human or animal sources. Biotechnology, bioengineering, and the use of cell banks have enabled the large-scale and reproducible production of these naturally occurring biologically derived drugs ().

The size and complexity of protein and nucleic acid drugs require extensive design and engineering of the manufacturing and control processes to produce the drug in large quantities with consistent quality. The size of a protein or peptide drug can range from a few hundred to several hundred thousand daltons. The three-dimensional structure of a protein or peptide drug is important for its pharmacodynamic activity, so the corresponding specific primary amino acid, secondary (alpha or beta helix), tertiary (special relationship of secondary structures), or even quaternary orientation of subunits must be considered. A biotechnology-derived drug (also referred to as a *biologic drug* or *biopharmaceutical*) must be designed such that the structure is stable, reproducible, and accurate during manufacture, storage, and administration. The manufacturing process and product are intricately linked. Small changes in the manufacturing process may affect the sequence of the resulting protein, but are more likely will affect the structure, yield, or activity of the protein. Therefore, pharmaceutical controls and testing must be carefully designed, controlled, and monitored, and must also be able to distinguish minor chemical or structural changes that could affect the safety or efficacy in the product during each of these stages.

Drug delivery of biologics can be a problem for therapeutic use because the protein drug must reach the site of action physically and structurally intact. Biologic drugs are notoriously unstable in plasma and the gastrointestinal tract, so modifications to improve drug delivery or stability are often required. Currently, most biologic drugs are generally too unstable for oral delivery and must usually be administered by parenteral routes. However, other, nonparenteral routes of administration, such as intranasal and inhalation, are being investigated for biologic drug delivery. Fortunately, because many of these recombinant protein drugs are designed to act extracellularly, transmembrane delivery may not be required once the drug reaches the plasma.

Monoclonal Antibodies

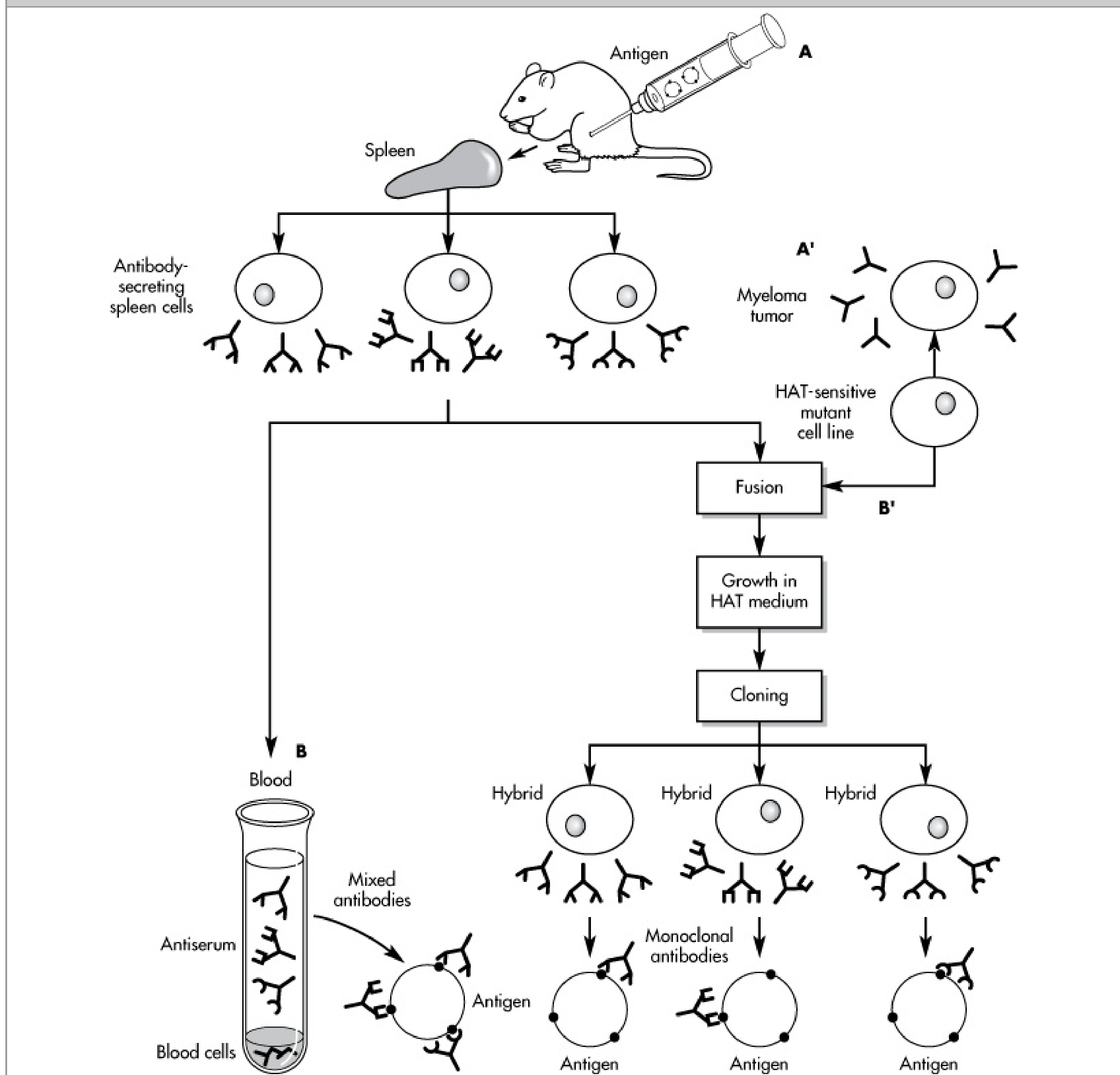
Another new class of protein drugs is *monoclonal antibodies* (mAbs). Antibodies are produced by the body's immune system for specific recognition and removal of foreign bodies. The power of mAbs lies in their highly specific binding of only one antigenic determinant. As a result, mAb drugs, targeting agents, and diagnostics are creating new ways to treat and diagnose previously untreatable diseases and to detect extraordinarily low concentrations of protein or other molecules ().

Table 18.2 Applications of Monoclonal Antibodies
Cancer treatment
mAbs against leukemia and lymphomas have been used in treatment with variable results. Regression of tumor is produced in about 25%, although mostly transient.
Imaging diagnosis
mAbs may be used together with radioactive markers to locate and visualize the location and extent of the tumors.
Target-specific delivery
mAbs may be conjugated to drugs or other delivery systems such as liposomes to allow specific delivery to target sites. For example, urokinase was conjugated to an antifibrin mAb to dissolve fibrin clots. The carrier system would seek fibrin sites and activate the conversion of plasmogen to plasmin to cause fibrin to degrade.
Transplant rejection suppression
In kidney transplants, a mAb against CD3, a membrane protein of cytotoxic T cells that causes a rejection reaction, was very useful in suppressing rejection and allowing the transplant to function. The drug was called OKT3. mAbs are also used for kidney and bone marrow transplants.

Theoretically, an almost infinite amount and number of antibodies can be produced by the body to respond immunologically to foreign substances containing antigenic sites. These antigenic sites are usually on protein molecules, but nonprotein material or *haptens* may be conjugated to a protein to form an antigen. Periodic injections of an antigen into an animal result in production of antibodies that bind a site or sites on that antigen. The serum of the animal will also contain antibodies to antigens to which the animal has been previously exposed. Though these mixtures of antibodies in the serum (*polyclonal antibodies*) are too impure for therapeutic use, they can be used for diagnostic immunoassays.

In contrast to polyclonal antibodies, mAbs are preparations that contain many copies of a single antibody that will therefore bind to and only detect one antigenic site. The purity of these preparations make them very useful as both diagnostics and as new therapeutic agents. However, the techniques for the preparation of mAbs are quite complicated. In mAb production, normal antibody-producing cells, such as a mouse spleen cell, are fused with a myeloma cell and allow the hybrid cells (*hybridoma*) to grow in a test tube. The nonfused cells will die, the myeloma cells will be selectively destroyed with an antitumor drug such as aminopterin (), whereas the hybridoma cells will continue to grow. Each hybridoma cell is then separated into a separate growth chamber or well in which they are allowed to multiply. Each cell and its clones in the respective growth chamber will make antibodies to only one antigen (mAb). The cells producing the desired antibody are selected by testing each well for mAb binding to the desired antigen. The desired cells (clones) are then expanded for mAb production. Since the resulting mAb is of murine origin, often genetic engineering is used to "humanize" the mAb, thus minimizing an immune response to the therapeutic mAb.

Figure 18-1.Monoclonal antibody production.



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A. A mouse is immunized with an antigen bearing three antigenic determinants (distinct sites that can be recognized by an antibody). Antibodies to each determinant are produced in the spleen. One spleen cell produces a single type of antibody. A spleen cell has a finite lifetime and cannot be cultured indefinitely *in vitro*. **B.** In the mouse, the antibody-producing cells from the spleen secrete into the blood. The liquid portion of the blood (serum) therefore contains a mixture of antibodies reacting with all three sites on the antigen (antiserum). **A'.** A mutant cell derived from a mouse myeloma tumor of an antibody-producing cell that has stopped secreting antibody and is selected for sensitivty to the drug aminopterin (present in HAT medium). This mutant tumor cell can grow indefinitely *in vitro* but is killed by HAT medium. **B'.** The mutant myeloma cell is fused by chemical means with spleen cells from an immunized mouse. The resulting hybrid cells can grow indefinitely *in vitro* due to properties of the myeloma cell parent and can grow in HAT medium because of an enzyme provided by the spleen cell parent. The unfused myeloma cells die because of their sensitivity to HAT, and unfused spleen cells cannot grow indefinitely *in vitro*. The hybrid cells are cloned so that individual cultures are grown from a single hybrid cell. These individual cells produce a single type

of antibody because they derive from a single spleen cell. The monoclonal antibody isolated from these cultures is specific for only one antigenic determinant on the original antigen.

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Monoclonal antibodies may be used therapeutically to neutralize unwanted cells or molecules. Several mAbs with proven indications are listed in , , and . Monoclonal antibodies are used as antivenoms (CroFab), for overdose of digoxin (DigiFab), or to neutralize endotoxin (Nebacumab, investigative) or viral antigen (Nabi-HB). Nebacumab is a human IgM mAb (HA-1A) with specificity for the lipid designed for septic shock treatment. Monoclonal antibodies (mAb) are named by a source identifier preceding "-mab," e.g., -**u**mab (human), -**o**mab (mouse), -**zu**mab (humanized), and -**xi**mab (cl.meric). Other common indications for mAb drugs include imaging (ProstaScint, Myocint, Verluma), cancer (Campath, Ontak, Zevalin, Rituxan, Herceptin), rheumatoid arthritis (Humira, Remicade), and transplant immunosuppression (Simulect, Thymoglobulin). Monoclonal antibodies are also used for more novel indications. For example, Abciximab (c7E3 Fab, ReoPro) is a chimeric mAb Fab (humanized) fragment specific for platelet glycoprotein IIb-IIIa receptors. This drug is extremely effective in reducing fatalities (0.50%) in subjects with unstable angina after angioplasty treatment.

Table 18.3 Approved Monoclonal Antibody Drugs and In-Vivo Diagnostics

mAb Product (Trade Name)	Target		Indication
Abciximab (ReoPro)	Platelet surface glycoprotein	Half-life < 10 min	Unstable angina Coronary angioplasty or atherectomy (PCTA) Antiplatelet prevention of blood clots
Adalimumab (Humira)	Tumor necrosis factor	V_d = 4–6 L; Cl = 12 mL/hr; half-life = 2 wk	Rheumatoid arthritis
Alefacept (Amevive)	CD2 (LFA) on lymphocytes	Half-life = 270 hr; Cl = 0.25 mL/kg/hr; V_d = 94 mL/kg	Psoriasis
Alemtuzumab (Campath)	CD52 on blood cells	Half-life = 12 d	B-cell chronic lymphocytic leukemia
Antithymocyte globulin (rabbit) thymoglobulin	T-lymphocyte antigens	Half-life = 2–3 d	Acute rejection in renal transplant patients
Basiliximab (Simulect)	Interleukin-2	Half-life = 7.2 d; V_d = 8.6 L; Cl = 41 mL/hr	Renal transplantation immunosuppression
Capromab pendetide (ProstaScint)	Prostate glycoprotein	Half-life = 67 hr; Cl = 42 mL/hr; V_d = 4 L	Diagnosing imaging agent in prostate cancer
Daclizumab (Zenapax)	Interleukin-2 receptor	Half-life = 20 d; Cl = 15 mL/hr; V_d = 6 L	Renal transplants immunosuppression
Denileukin diftitox (Ontak)	Interleukin-2 mAb conjugate to diptheria toxin	Half-life = 70–80 min; Cl = 1.5–2 mL/min/kg; V_d = 0.06–0.08 L/kg	Cutaneous T-cell lymphoma
Digoxin Immune Fab —Ovine (DigiFab)	Digoxin	Half-life = 15–20 hr; V_d = 0.3–0.4 L/kg	Digoxin toxicity or overdose
Etanercept (Enbrel)	Tumor necrosis factor receptor	Half-life = 115 hr; Cl = 89 mL/hr	Rheumatoid arthritis
Hepatitis B immune globulin—human (Nabi-HB)	Hepatitis B	Half-life = 25 d; Cl = 0.4 L/d; V_d = 15 L	Acute exposure to hepatitis B
Ibritumomab tiuxetan (Zevalin)	CD28 on B cells	Half-life = 30 hr	Follicular or transformed B-cell non-Hodgkin's lymphoma
Imciromab pentetate (Myoscint)	Myosin	Half-life = 20 hr	Imaging agent for detecting myocardial injury
Infliximab (Remicade)	Tumor necrosis factor	Half-life = 9.5 d; V_d = 3 L	Crohn's disease
			Rheumatoid arthritis
Nofetumomab (Verluma)	Carcinoma-associated antigen, Tc^{99m} labeled	Half-life = 10.5 hr	Detection of small cell lung cancer

Muromonab-CD3 (Orthoclone OKT3)	CD3 on T cells		Reversal of acute kidney transplant rejection
Palivizumab (Synagis)	RSV antigens	Half-life = 197 hr; *Cl* = 0.33 mL/hr/kg; V_d = 90 mL/kg	RSV disease
Rituximab (Rituxan)	CD20 on B cells	Half-life = 60 hr	Follicular, B-cell non-Hodgkin's lymphoma
Trastuzumab (Herceptin)	Human epidermal growth factor receptor	Half-life = 1.7–12 d; V_d = 44 mL/kg	Metastatic breast cancer whose tumors overexpress the HER2 protein

Monoclonal antibodies can also target and deliver toxins specifically to cancer cells and destroy them while sparing normal cells (see below), and they are important detectors used in laboratory diagnostics.

Gene Therapy

Gene therapy refers to a pharmaceutical product that delivers a recombinant gene to somatic cells *in vivo* (). In turn, the gene within the patients' cell produces a protein that has therapeutic benefit to the patient. The therapeutic approach in gene therapy is often the restoration of defective biologic function within cells, as is frequently seen in inherited disorders and cancer.

Gene therapy has been applied to the inherited disorder cystic fibrosis, in which patients have a defective chloride ion channel gene, resulting in chloride ion channel abnormalities. Although improved therapy has transformed cystic fibrosis from a disease characterized by death in early childhood to a chronic illness, there still is no cure for the disease and most patients eventually succumb to infections of the airways and lung failure. The aim of gene therapy for these patients is to deliver a "normal" chloride ion channel gene to the cells of cystic fibrosis patients and restore chloride transport in cells. More information on gene therapy of cystic fibrosis has been published by the National Institutes of Health (www.niddk.nih.gov/health/endo/pubs/cystic/cystic.htm).

Gene therapy faces several challenges despite over a decade of research and development. These challenges include gene delivery, sufficient extent and duration of stable gene expression, and safety. Because the gene coding the therapeutic protein (*transgene*) must also contain gene control regions such as the promoter, the actual rDNA (*recombinant DNA*) to be delivered to target cells' nucleus can easily be 10–20 kilobases (kb) in size.

Two main approaches have been used for *in-vivo* delivery of rDNA. The first is a virus-based approach that involves replacing viral replicative genes with the transgene, then packaging the rDNA into the viral particle. The recombinant virus can then infect target cells, and the transgene is expressed, though the virus is not capable of replicating. Both retroviruses, RNA viruses that have the ability to permanently insert their genes into the chromosomes of the host cells, and DNA viruses (which remain outside host chromosomes) have been used successfully in viral gene delivery. Most of the gene therapy trials worldwide involve the use of such viral delivery systems.

In addition to viral delivery systems (*vectors*), nonviral approaches have been used with some success for *in-vivo* gene delivery. The transgene is engineered into a plasmid vector, which contains gene-expression control regions. These naked DNA molecules may enter cells and express product in some cell types, such as muscle cells. This naked DNA delivery technique is being tested as possible DNA vaccines, in which the muscle cells produce small amounts of antigen that stimulate immunity to the antigen. However, usually either cationic or a fusogenic liposome delivery systems (see below) is required in most other cell types to produce measurable levels of transgene expression. Both types of lipid vesicles or particles result in intracellular delivery of DNA to cells.

An alternative to direct *in-vivo* delivery is a cell-based approach that involves the administration of transgenes to cells that have been removed from a patient. For example, cells (usually bone marrow cells) are removed from the patient; genes encoding a therapeutic product are then introduced into these cells *ex vivo* using a viral or nonviral delivery system, and then the cells are returned into the patient. The advantage of *ex-vivo* approaches is that systemic toxicity of viral or nonviral delivery systems is avoided.

Effective gene therapy depends on several conditions. The vector must be able to enter the target cells efficiently and deliver the corrective gene without damaging the target cell. The corrective gene should be stably expressed in the cells, to allow continuous production of the functional protein. Neither the vector nor the functional protein produced from it should cause an immune reaction in the patient. It is also difficult to control the amount of functional protein produced after gene therapy, and excess production of the protein could cause side effects, although insufficient production is more typically observed. Additional problems in gene therapy include the physical and chemical properties of DNA and RNA molecules, such as size, shape, charge, surface characteristics, and the chemical stability of these molecules and delivery systems. *In-vivo* problems may include bioavailability, distribution, and uptake of these macromolecules into cells. Moreover, naked DNA molecules are rapidly degraded in the body ().

Antisense Drugs

Antisense drugs are drugs that seek to block DNA transcription or RNA translation in order to moderate many disease processes. Antisense drugs consist of nucleotides linked together in short DNA or RNA sequences known as *oligonucleotides*. Oligonucleotides are designed knowing the target DNA/RNA to bind to specific DNA or RNA sequences or regions (eg, messenger RNA) to block transcription or translation of that targeted protein. An oligonucleotide that binds complementary ("sense") mRNA sequences and blocks translation is referred to as *antisense*. To further stabilize the drug, many chemical modifications have been made to the oligonucleotide structure. The most common modification used involves substitution of a nonbridging oxygen in

the phosphate backbone with sulfur, resulting in a phosphorothioate-derived antisense oligonucleotide. Some of these drugs have been designed to target viral disease and cancer cells in the body. Vitravene (ISIS Pharmaceuticals), an oligonucleotide targeted to cytomegalovirus, was the first antisense oligonucleotide drug approved by the U.S. Food and Drug Administration (FDA).

For this approach to be useful, the etiology and genetics of the disease must be known. For example, in the case of viral infection, known sequences belonging to vital genes can be targeted and inhibited by antisense drugs. Many antisense sequences are usually tested to find the best candidate, since intra- and intermolecular interactions can affect oligonucleotide activity and delivery. Though oligonucleotides are relatively well internalized compared to rDNA molecules, cellular uptake is often low enough to require delivery systems, such as liposomes. Antisense and gene therapy approaches have also been combined using viral vectors to deliver an antisense sequence. In this case, the transgene is transcribed into an mRNA molecule that is antisense and therefore binds to the target mRNA. The resulting RNA:RNA interaction is high affinity and results in inhibition of translation of that mRNA molecule.

DRUG CARRIERS AND TARGETING

Formulation and Delivery of Protein Drugs

Advances in biotechnology have resulted in the commercial production of naturally produced active drug substances for drug therapy (). These substances hold great potential for more specific drug action with fewer side effects. However, many naturally produced substances are complex molecules, such as large-molecular-weight proteins and peptides. Conventional delivery of protein and peptide drugs is generally limited to injectables and implantable dosage forms. Insulin pumps for implantation have been developed for diabetes, for precise control of sugar levels.

Formulating protein drugs for systemic use by oral, or even any extravascular, route of administration is extremely difficult due to drug degradation and absorption from the site of administration. There are several requirements for effective oral drug delivery of protein and peptide drugs: (1) protection of the drug from degradation while in the harsh environment of the digestive tract, (2) consistent absorption of the drug in a manner that meets bioavailability requirements, (3) consistent release of the drug so that it enters the bloodstream in a reproducible manner, (4) nontoxicity, and (5) delivery of the drug through the GI tract and maintenance of pharmacologic effect similar to IV injection.

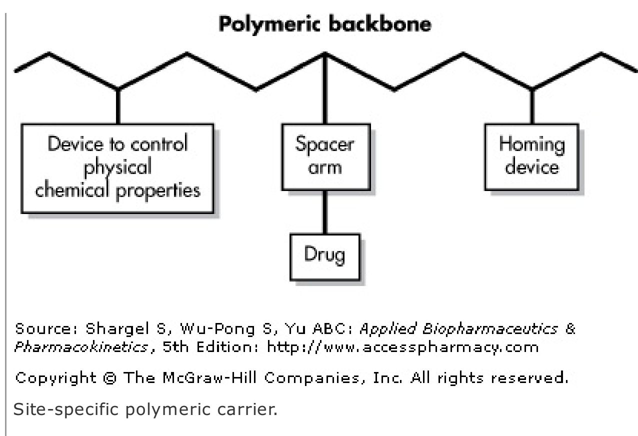
Designing, evaluating, and improving protein and peptide drug stability is considerably more complex than for small conventional drug molecules. A change in quaternary structure, such as aggregation or deaggregation of the protein, may result in loss of activity. Changes in primary structure of proteins frequently occur and include deamidation of the amino acid chains, oxidation of chains with sulfhydryl groups, and cleavage by proteolytic enzymes present throughout the body and that may be present due to incomplete purification. Because of protein drugs' complex structures, impurities are much harder to detect and quantify. In addition, proteins may be recognized as foreign substances in the body and become actively phagocytized by the reticuloendothelial system (RES), resulting in the inability of these proteins to reach the intended target. Proteins may also have a high allergenic or immunogenic potential, particularly when nonhuman genes or production cells are used.

Because of the many stability and delivery problems associated with protein and nucleic acid drugs, new delivery systems are being tested to improve their *in-vivo* properties. Carriers can be used to protect the drug from degradation, improve transport or delivery to cells, decrease clearance, or a combination of the above. In this chapter, carriers used for both small traditional drug and biopharmaceutical drug delivery are reviewed. Carriers may be covalently bound to the drug, where drug release is usually required for pharmacologic activity. Noncovalent drug carriers such as liposomes typically require uncoating of the drug for biologic activity to occur.

Polymeric Carriers and Conjugates

Polymers can be designed to include a wide range of physical and chemical properties and are popularly used in drug formulations because of their versatility. Polymers initially were used to prolong drug release in controlled-release dosage forms. The development of site-specific polymer or macromolecular carrier systems is a more recent extension of earlier research. The basic components of site-specific polymer carriers are (1) the polymeric backbone (), (2) a site-specific component (homing device) for recognizing the target, (3) the drug covalently attached to the polymer chain, and (4) functional chains to enhance the physical characteristics of the carrier system. Improved physical characteristics may include improved aqueous solubility. In the case of polymeric prodrugs, a spacer group may be present, bridging the drug and the carrier. The spacer chain may influence the rate at which the drug will hydrolyze from the prodrug system. At present, most site-specific polymeric drug carriers are limited to parenteral administration and primarily utilize soluble polymers.

Figure 18-2.



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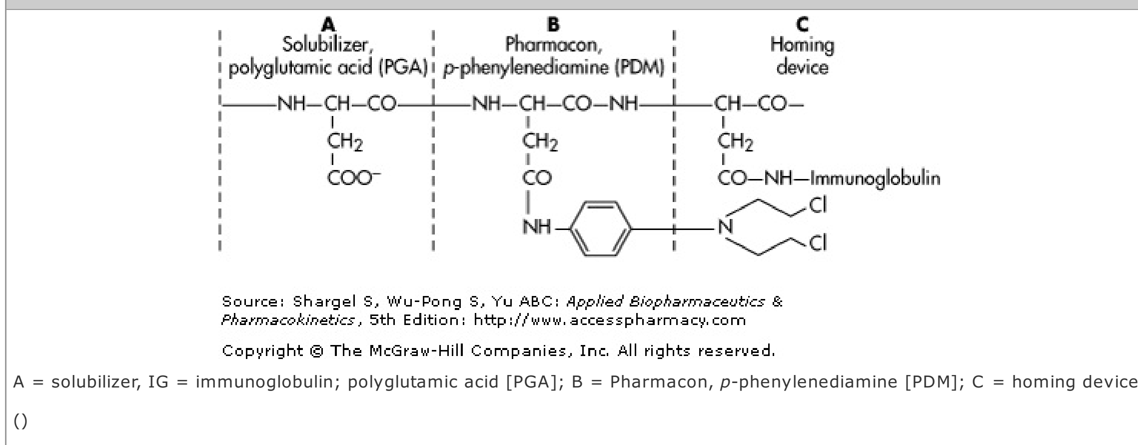
Site-specific polymeric carrier.

Positively charged polymers such as polyethylenediamine (PEI), polylysine, and chitosan () are used in noncovalent complexes for macromolecular drugs, such as gene or oligonucleotide therapy. For example, polymer:DNA complexes improve DNA delivery to cells in part by providing some protection from nuclease degradation *in vivo*. An added advantage of complexed cationic polymers is that targeting agents such as receptor ligands can be covalently attached to the polymer rather than the drug to provide cell-specific targeting. Cationic polymer use is limited because of toxicity of the polymer and dissociation of the complex *in vivo*.

Figure 18-3.



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A = solubilizer, IG = immunoglobulin; polyglutamic acid [PGA]; B = Pharmacon, *p*-phenylenediamine [PDM]; C = homing device.

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Polymers may also be covalently conjugated to drugs to improve their solubility or pharmacokinetic properties. Polymers with molecular weights greater than 30–50 kDa bypass glomerular filtration, thereby extending the duration of drug circulation in the body. Polyethylene glycol (PEG) is used to improve the clearance of some drugs, such as adenosine deaminase (PEG-ADA), filgrastim (Neulasta), interferon (PEG-Intron and PEGASYS), and asparaginase (Oncospar). Daunomycin has been linked to dextran, resulting in improved drug activity in animal studies (). Dextrans are large polysaccharide molecules (MW 2000 to 1 million Da) with good water solubility, stability, and low toxicity. Drugs with a free amino or hydroxyl group may be linked chemically to hydroxyl groups in dextrans by activation of the dextran with periodate, azide, or other agents.

The molecular weight of the polymer carrier is an important consideration in designing these dosage forms. Generally, large-molecular-weight polymers have longer residence time and diffuse more slowly. However, large polymers are also more prone to capture by the reticuloendothelial system. To gain specificity, a monoclonal antibody, a recognized sugar moiety, or a small cell-specific ligand may be incorporated as a targeting agent into the delivery system. For example, exposed galactose residues are recognized by hepatocytes; whereas mannose or L-fructose is recognized by surface receptors in macrophages. HMPA [N-(2-hydroxypropyl)methacrylamide] is commonly used in drug conjugates because the polymer can be modified with monosaccharides that act as targeting agents for cells expressing the appropriate receptor (). Similarly, a three-way conjugate consisting of mitomycin C, a monoclonal antibody (A7), and dextran (MW 70,000 Da) as the intermediate carrier resulted in a 10-fold increased activity against colon cancer cells due to improved target specificity ().

In addition to use as regular carriers, polymers may also be formulated into *microparticles* and *nanoparticles*. In such delivery systems, the therapeutic agent is encapsulated within a biodegradable polymeric colloidal particle that is in the micrometer or nanometer size range, respectively. Micro- and nanosphere formulations are useful for solubilizing poorly soluble drugs, improving oral bioavailability, protecting against degradation, or providing sustained drug delivery. The small size of nanospheres generally allows good tissue penetration while providing protection or sustained release.

The size of the microsphere and nanosphere has a profound impact on an encapsulated drugs' *in-vivo* properties and disposition. At over 12 µm, particles are lodged in the capillary bed at the site of the injection. From 2 to 12 µm, particles are retained at the lung, spleen, or liver. Particles less than 0.5 µm (500 nm) deposit into the spleen and bone marrow. In gene therapy, particles smaller than 100 nm demonstrate higher gene expression *in vitro* compared to larger particles (). Though some peptides and nucleic acids have been successfully formulated into nanospheres, protein denaturation and degradation can be significant

during encapsulation.

Cyclodextrins (CDs) are also used to improve stability, delivery, and water solubility of drugs. The lipophilic cavity of CDs typically contains the therapeutic agent, while the exterior of the CD molecule is hydrophilic and allows solubilization of the complex. Many folded proteins and nucleic acids are too large to be completely included into the CD cavity and can result in protein denaturation. However, CDs have been used to solubilize and stabilize several proteins and peptides ().

Albumin

Albumin is a large protein (MW 69,000 Da) that is distributed in the plasma and extracellular water. Albumin has been experimentally conjugated with many drugs to improve site-specific drug delivery. Methotrexate, cytosine arabinoside, and 6-fluorodeoxyuridine have each been conjugated with albumin. Methotrexate albumin conjugates may increase the duration of drug action after conjugation (). In general, the distribution of albumin is not site specific. Because albumin will concentrate in the liver, 5-fluorodeoxyuridine (Amanitin) albumin complex has been used experimentally to deliver drug to the Kupffer cells in the liver for treatment of ectromelia virus ().

Lipoproteins

Lipoproteins are lipid protein complexes in the blood involved in the circulation and distribution of lipids in the body. The lipid components are polar phospholipids and cholesterol. Because of their various sizes, lipoproteins have been classified according to molecular weight based on centrifugation: (1) high-density lipoprotein (HDL, MW 300,000–600,000 Da), (2) low-density lipoprotein (LDL, MW 2.3×10^6 Da), (3) very-low-density lipoprotein (VLDL, MW 10×10^6 Da), and (4) chylomicrons (MW 10^9 Da). Low-density lipoproteins enter the cell by a receptor-mediated pathway through the process of endocytosis. Endocytosis is a potential means of transporting drugs into the cell in which the lipoprotein–drug complex is hydrolyzed by intracellular lysosomal enzymes, releasing the active drug within the vesicle.

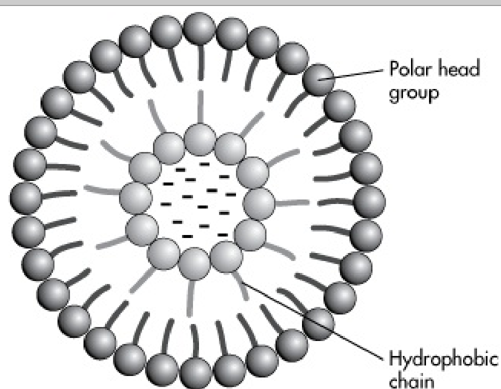
More research is needed on the use of lipoproteins for drug targeting. For example, lipophilic drugs may be dissolved within the core of the lipoprotein. After oral administration, fatty substances incorporated into the chylomicron formed in the gastrointestinal tract may be absorbed into the lymphatic system.

Liposomes

Liposomes have an aqueous, drug-containing interior surrounded by an exterior lipid bilayer, and typically range in size from 0.5 to 100 µm. Liposomes have been used successfully to reduce side effects of antitumor drugs and antibiotics. For example, doxorubicin liposomes have reduced cardiotoxicity and emetic side effects. Amphotericin B may have reduced nephrotoxicity side effects when formulated with liposomes. An innovative liposome-related product (Abelcet) consists of amphotericin B complexed with two phospholipids, L-a-dimyristoyl-phosphatidylcholine and L-a-dimyristoylphosphatidylglycerol (Liposome Company, www.lipo.com). The lipid drug complex releases the drug at the site of infection and reduces renal toxicity of amphotericin B without altering its antifungal activity. A more representative liposome product is AmBisome (NeXstar), which consists of very fine liposomes of amphotericin B. The product significantly reduces the side effects of amphotericin B. Other investigative liposomal products include TLC-C-53 (liposomal prostaglandin E1) for the treatment of acute respiratory distress syndrome (ARDS). Daunorubicin citrate liposomal (DaunoXome, NeXstar) is an aqueous solution of the citrate salt of the antineoplastic daunorubicin encapsulated within lipid vesicles. The distearoylphosphotidylcholine and cholesterol (2:1 molar ratio) liposome formulation in DaunoXome attempts to maximize the selectivity of daunorubicin into solid brain tumors. Once in the tumor, daunorubicin is released and exerts its antineoplastic activity. Liposome formulations have also been prepared with gentamicin, cisplatin, and other drugs.

There are three general ways of preparing conventional liposomes: (1) phase separation, (2) spray or shear method through orifice, and (3) coacervation. The choice of method depends on the drug, the yield requirements, and the nature of the lipids. Formation of the liposome bilayer depends on the hydrophobic and hydrophilic orientation of the lipids ().

Figure 18-4.



Source: Shargel S, Wu-Pong S, Yu ABC: *Applied Biopharmaceutics & Pharmacokinetics*, 5th Edition: http://www.accesspharmacy.com

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Diagrammatic representation of a liposome showing polar head group and hydrophobic chain.

Liposomes have different electrical surface charges depending on the type of material used. Common anionic lipid materials are phosphatidyl choline and cholesterol. The phosphatidyl group is amphiphilic, with the choline being the polar group. This structure allows each molecule to attach to others through hydrophobic and hydrophilic interactions. Thermodynamically, liposomes are in equilibrium between different membrane conformations or structures (lipid polymorphism). Thus, some seemingly stable liposome systems exhibit leakage and generally do not have long shelf lives.

Liposomes can be engineered to be site specific. Generally, site specificity is conferred by the type of lipid or by inclusion of a targeting agent, such as a monoclonal antibody, into the liposome bilayer (see , below). Liposomes may be used to improve intracellular delivery, in which case the liposome must also be designed to fuse with the plasma or endosome membrane. Lipids or fusogenic peptides that facilitate membrane fusion, such as phosphatidyl ethanolamine or GALA and KALA peptides, respectively, have been used to improve liposome intracellular delivery. Cationic lipids, such as N-[1-(2, 3 dioleyloxy)propyl]-N,N, N-trimethylammonium chloride (DOTMA) or oleoyl-phoshphatidylethanolamine (DOPE), are also commonly used for *in-vitro* delivery of DNA. When cationic lipids are mixed with DNA, a particle forms from DNA:lipid charge interactions. The cationic lipid is believed to destabilize biological membranes resulting in improved intracellular DNA delivery. The *in-vivo* use of cationic lipids is limited by systemic toxicity due to the positive charge of the lipid.

TARGETED DRUG DELIVERY

Most conventional dosage forms deliver drug into the body that eventually reaches the site of action by distribution and passive diffusion. In addition, the drug also distributes to nontarget site tissues. Because of nonselective distribution, a much larger dose is given to the patient to achieve therapeutic concentrations in the desired tissue. However, drug action at nontarget sites may result in toxicity or other adverse reactions. Delivery systems that target the drug only to the desired site of drug action allow for more selective therapeutic activity. For biopharmaceuticals, selective and targeted drug therapy could result in a significant reduction in dose and cost.

Targeted drug delivery or *site-specific drug delivery* refers to drug carrier systems that place the drug at or near the receptor site. have classified site-specific drug delivery into three broad categories or drug targeting: (1) first-order targeting, which refers to drug delivery systems that deliver the drug to the capillary bed of the active site; (2) second-order targeting, which refers to the specific delivery of drug to a special cell type such as the tumor cells and not to the normal cells; and (3) third-order targeting, which refers to drug delivery specifically to the internal (intracellular) site of cells. An example of third-order drug targeting is the receptor-mediated entry of a drug complex into the cell by endocytosis followed by lysosomal release of the lysosomally active drug. Numerous techniques have been developed for site-specific delivery. Ideally, site-specific carriers guide the drug to the intended target site (tissues or organ) in which the receptor is located without exposing the drug to other tissues, thereby avoiding adverse toxicity. Much of the research in targeted drug delivery has been in cancer chemotherapy.

Site-specific drug delivery has also been characterized as passive or active targeting (). *Passive targeting* refers to the exploitation of the natural (passive) disposition profiles of a drug carrier, which are passively determined by its physicochemical properties relative to the anatomic and physiologic characteristics of the body. *Active targeting* refers to alterations of the natural disposition of a drug carrier, directing it to specific cells, tissues, or organs. One approach to active targeting is the use of ligands or monoclonal antibodies which can target specific cells. Monoclonal antibodies were discussed more fully earlier in this chapter. Active targeting employing receptor-mediated endocytosis is a saturable, nonlinear process that depends on the drug–carrier concentration, whereas passive targeting is most often a linear process over a large range of doses.

General Considerations in Targeted Drug Delivery

Considerations in the development of site-specific or targeted drug delivery systems include: (1) the anatomic and physiologic characteristics of the target site, including capillary permeability to macromolecules and cellular uptake of the drug (); (2) the physicochemical characteristics of the therapeutically active drug; (3) the physical and chemical characteristics of the carrier; (4) the selectivity of the drug–carrier complex; (5) any impurities introduced during the conjugation reaction linking the drug and the carrier that may be immunogenic, toxic, or produce other adverse reactions.

Target Site

The accessibility of the drug–carrier complex to the target site may present bioavailability and pharmacokinetic problems, which also include anatomic and/or physiologic considerations. For example, targeting a drug into a brain tumor requires a different route of drug administration (intrathecal injection) than targeting a drug into the liver or spleen. Moreover, the permeability of the blood vessels or biologic membranes to macromolecules or drug carrier complex may be a barrier preventing delivery and intracellular uptake of these drugs ().

Site-Specific Carrier

To target a drug to an active site, one must consider whether there is a unique property of the active site that makes the target site differ from other organs or tissue systems in the body. The next consideration is to take advantage of this unique difference so that the drug goes specifically to the site of action and not to other tissues in which adverse toxicity may occur. In many cases the drug is complexed with a carrier that targets the drug to the site of action. The successful application of these delivery systems requires the drug–carrier complex to have both affinity for the target site and favorable pharmacokinetics for delivery to the organ, cells, and subcellular target sites. An additional problem, particularly in the use of protein carriers, is the occurrence of adverse immunological reactions—an occurrence that is partially overcome by designing less immunoreactive proteins ().

Drugs

Most of the drugs used for targeted drug delivery are highly reactive drugs that have potent pharmacodynamic activities with a narrow therapeutic range. These drugs are often used in cancer chemotherapy. Many of these drugs may be derived from

biologic sources, made by a semisynthetic process using a biologic source as a precursor, or produced by recombinant DNA techniques. The drugs may be large macromolecules, such as proteins, and are prone to instability and inactivation problems during processing, chemical manipulation, and storage.

Targeting Agents

Properly applied, drug targeting can improve the therapeutic index of many toxic drugs. However, monoclonal antibodies (see discussion above) are not the "magic bullet" for drug targeting that many people had hoped. One difficulty encountered is that the large molecule reduces the total amount of active drug that can be easily dosed (ie, the ratio of drug to carrier). In contrast, conventional carriers that are not specific are often many orders of magnitude smaller in size, and a larger effective drug dose may be given more efficiently.

In addition to employing monoclonal antibodies in liposomes and other delivery systems as described above, mAbs may be conjugated directly to drugs. The resulting conjugate can theoretically deliver the drug directly to a cell that expresses a unique surface marker. For example, a tumor cell may overexpress the interleukin-2 receptor. In this case, a cytotoxic molecule such as recombinant diptheria toxin is coupled to a mAb specific for the interleukin-2 receptor (Ontak). The conjugate delivers the toxin preferentially to these tumor cells. An overall tumor response rate for Ontak is 38%, with side effects including acute hypersensitivity reaction (69%) and vascular leak syndrome (27%). Zolimomab aritox (Orthozyme-CD5, Xoma/Ortho Biotech) is an investigational immunoconjugate of monoclonal anti-CD5 murine IgG and the ricin A-chain toxin. This conjugate is used in the treatment of steroid-resistant graft-versus-host disease after allogeneic bone marrow transplants for hematopoietic neoplasms, such as acute myelogenous leukemia. Myoscint is an ^{111}In-labeled mAb targeted to myosin that is used to image myocardial injury in patients with suspected myocardial infarction. An immune response to mAb drugs may develop, since mAbs are produced in mouse cells. Recent efforts to engineer or "humanize" mAbs have produced a new generation of molecules that are less immunogenic.

Oral Immunization

Antigens or fragmented antigenic protein may be delivered orally and stimulate gut-associated lymphoid tissue (GALT) in the gastrointestinal tract. This represents a promising approach for protecting many secretory surfaces against a variety of infectious pathogens. Immunization against salmonella and *Escherichia coli* in chickens was investigated for agricultural purpose. Particulate antigen delivery systems, including several types of microspheres, have been shown to be effective orally inducing various types of immune response. Encapsulation of antigens with mucosal adjuvants can protect both the antigen and the adjuvant against gastric degradation and increase the likelihood that they will reach the site of absorption.

PHARMACOKINETICS OF BIOPHARMACEUTICALS

The unusual nature of biopharmaceuticals compared to traditional drugs presents new development challenges for scientists in the biotechnology industry. Because of the size and complexity of biopharmaceuticals, stability and delivery are major developmental issues with these new drugs. The prerequisite of the maintenance of higher-order structure adds a new dimension to formulation, drug delivery, and stability testing of biologic drugs. Pharmacokinetic studies are often complicated by bioanalytic challenges, since preservation of primary structure or an isotope label alone does not necessarily coincide with biologic activity.

Once in the body, protein and nucleic acid drugs are subject to rapid degradation by endogenous proteases and nucleases that are present in the serum, tissues, and cells. Unmodified phosphodiester DNA and RNA are extremely labile in the body, with half-lives of the order of a few minutes. report that naked DNA clearance in rats is rapid and depends on the conformation of the plasmid: supercoiled, open circular, versus linear. Many of the early recombinant protein drugs also have half-lives of the order of a few minutes, such as Alteplase (Activase) and interleukin-2 (Proleukin) (). However, if immediate stability or immunigenicity concerns can be remedied by chemical modification or bioengineering, the biopharmaceutical may be large enough to escape glomerular filtration and enjoy a prolonged circulation in the body ().

The size and generally hydrophilic nature of the nucleic acid and protein molecules also often precludes the use of diffusional and paracellular transport pathways available to small drug molecules. The capillary wall in most organs and tissues limits passage of macromolecules such as albumin. A typical vector is 20–150 nm, and monoclonal antibodies are composed of four polypeptide chains (over 1200 amino acids total). Such compounds would be expected to have limited diffusional access to most tissues, except the liver, spleen, bone marrow, and tumor tissues, which have higher vascular permeability. As a result, the volume of drug distribution is often smaller for the larger protein and nucleic acid drugs because of vascular confinement or binding to specific tissues. Indeed, the volume of distribution for some of these drugs approximates plasma volume: the apparent volume of distribution at steady state of the mAb Nebacumab is 0.11 ± 0.03 L/kg (), and of Simulect is approximately 7.5 L.

Because of the stability and distribution limitations of large biologic drugs, delivery systems such as conjugates, nanoparticles, liposomes, and viral vectors as described above have been used to improve activity and delivery. The pharmacokinetics of recombinant viral gene delivery systems have been difficult to measure because of the relatively low doses given and often inefficient transgene expression. As a result, gene expression and transgene persistence in tissues are used to determine pharmacokinetic profiles (). Nonviral and naked DNA delivery systems are relatively well characterized in comparison to viral delivery systems. , using polymerase chain reaction (PCR), demonstrate that intramuscular or cutaneous injection of a DNA vaccine resulted in gene expression primarily in surrounding tissues unless extremely high doses were administered. Nomura et al (1998) similarly showed that intratumoral injection of plasmid complexed with cationic lipid resulted in primarily local expression.

Liposome delivery systems are fairly well characterized in terms of their pharmacokinetic properties. Liposome encapsulation may reduce the V_D (), and may () or may not () improve upon DNA half-life by several hours. However, lipid delivery systems are also

rapidly cleared by the mononuclear phagocyte system (spleen and liver) unless injected intratumorally (). In addition, liposomes may enhance an immune response to the drug and complement activation, also resulting in rapid clearance.

Alternatively, liposomes can be designed to evade phagocyte detection and improve circulation time by coating with polyethylene glycol (PEG), which minimizes opsonin-dependent clearance. *In vivo,* the PEG provides a "bulky" headgroup that serves as a barrier to prevent interaction with the plasma opsonins. The hydrated groups sterically inhibit hydrophobic and electrostatic interaction of a variety of blood components at the liposome surface, thereby evading recognition by the reticuloendothelial system. An example of this concept is the Stealth liposome, which led to the marketing of the PEG-ylated liposomal doxorubicin, Doxil in the United States. Liposomal PEG-ylation can reduce the volume of distribution and extend the half-life of a drug such as doxorubicin (). Optimal formulation of a PEG-ylated liposome can improve liposome stability from 1% to 31% of dose remaining in the body at 24 hours postinjection ().

The pharmacokinetics of a liposomal formulation can be different from those of a nonliposomal product given by the same route of administration. For new liposome products, the FDA (draft document, see www.fda.gov/cder/guidance/2191dft.pdf) recommends a comparative mass balance study be performed to assess the differences in systemic exposure and pharmacokinetics between liposome and nonliposome drug products when (1) the two products have the same active moiety, (2) the two products are given by the same route of administration, and (3) one of the products is already approved for marketing. If satisfactory mass balance information is already available for the approved drug product, a limited mass balance study can be undertaken for the new drug product. Comparison of the absorption, distribution, metabolism, and excretion (ADME) of the liposome and nonliposome drug product forms should be made, using a crossover or a parallel noncrossover study design that employs an appropriate number of subjects.

BIOEQUIVALENCE OF BIOTECHNOLOGY-DERIVED DRUG PRODUCTS

The dosage form or formulation of a drug product may change during the course of drug development. The initial drug formulation used in early clinical studies (eg, Phase I/II) may not be the same formulation as the drug formulation used in later clinical trials (Phase III) or the marketed formulation. The demonstration of the bioequivalence of biotechnology products may be difficult (). After patent expiration, pharmaceutical manufacturers may develop a generic equivalent. Currently, there is no general scientific agreement as to how to demonstrate bioequivalence of biotechnology-derived drug products (generic biologics). See for a more detailed on bioequivalence.

FREQUENTLY ASKED QUESTIONS

1. What is the most frequent route of administration of biologic compounds?

2. What is the effect of glycosylation on the activity of a biologic compound? Give an example.

3. What are the major differences in drug distribution and elimination between conventional molecules and biotechnological compounds?

4. What is meant by targeted drug delivery? How does gene therapy differ from targeted drug delivery?

5. Why are macromolecular carrier systems used for targeted drug delivery?

6. What are monoclonal antibodies? What advantages do monoclonal antibodies have as carriers for site-specific drug delivery?

LEARNING QUESTIONS

1. Explain why most drugs produced by biotechnology cannot be given orally. What routes of drug administration would you recommend for these drugs? Why?

2. What is meant by site-specific drug delivery? Describe several approaches that have been used to target a drug to a specific organ.

3. Doxorubicin (Adriamycin) is available as a conventional solution and as a liposomal preparation. What effect would the liposomal preparation have on the distribution of doxorubicin compared to an injection of the conventional doxorubicin injection?

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