Recent Advances in Drug Delivery Systems: Polymeric Prodrugs

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During the past decade, scientists have focused their attention on developing site-specific drug delivery systems, and various polymers have shown promising results in this area. Ringsdorf's model of polymeric prodrugs depicts the ideal drug delivery system — one which has all the desired physicochemical properties and delivers the drug at the desired tissue or intracellular region. This article discusses the various polymers used for site-specific drug delivery and considers the advantages and disadvantages of this type of delivery system.

In addition to bioavailability and pharmacokinetics, site-specific drug delivery has been a topic of interest to pharmaceutical scientists during the past ten years. By delivering a drug at the site of action, these "targeted" delivery systems improve the drug's therapeutic effectiveness and minimize its toxic effects on other tissues. Drug delivery systems generally are classified as carrier systems, mechanical pumps, or prodrugs. The category of carrier system is divided into three major groups: macromolecular drug delivery systems, particulate systems, and cellular drug carriers.[1]

Proteins such as antibodies and lipoproteins; liposomes; synthetic polymers; and polysaccharides, such as dextran and inulin, are the various types of macromolecules used as drug delivery systems.[2-4] Polymers have been used extensively in these systems, including systems such as nanoparticles, microcapsules, laminates, matrices, and microporous powders.[5-11] In all these delivery systems, the drug is merely dispersed or incorporated into the system without the formation of a covalent bond between the drug and polymer. This article will discuss only those polymeric drug delivery systems in which a drug is covalently bonded to a polymeric backbone.

Because the molecular weight of polymeric drug delivery systems is very high, such systems are often referred to as *macromolecular carrier systems*. Prodrugs, which are derivatives of drugs, undergo biotransformation to release the drug in vivo. The use of prodrugs as drug dosage forms depends on the ability of the body to release the drug in the body.[12] Although the majority of polymer–drug conjugate systems have no biological activity, all such systems release the conjugated drug in vivo. For this reason, these polymeric macromolecular systems have been called *polymeric prodrug systems*.

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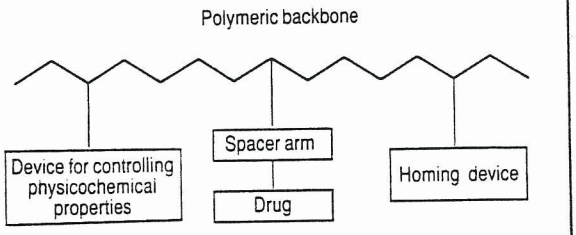

Figure 1: Ringsdorf's model of polymeric prodrugs.

Ringsdorf's Model

A schematic diagram of Ringsdorf's model is shown in Figure 1.[13] The system has a polymer backbone, which can be a homopolymer or a heteropolymer, depending on the constituents of the carrier polymer. The backbone contains three essential units; the first is a device for controlling the physicochemical properties of the entire macromolecule, which mainly involves the hydrophilic–lipophilic balance, the electric charge, and the solubility of the system.[14-15] The second and most important functional group is the drug itself. The drug must be covalently bonded to the polymer and must remain attached to it until the macromolecule reaches the desired site of action. The drug also must be detached from the parent polymer at the site of action. The release of drug takes place by hydrolysis or by specific enzymatic cleavage of the drug–polymer bond. In many cases, the drug is attached to the polymer through a spacer molecule, which is an amino acid or other simple molecule that can be cleaved or hydrolyzed at the desired site in the body.

The choice of drug for use in this system is based on three cri-

teria. First, only potent drugs can be used because there is a restriction on the amount of drug that can be administered. Second, the drug should have a functional group by which it can bind with the polymer backbone directly or by means of a spacer molecule. Third, the drug must be sufficiently stable and should not be excreted in this conjugate form until it is released at the desired site. The third functional unit of the polymer backbone is known as the "homing device." This unit guides the entire drug–polymer conjugate to the targeted tissue. Although not much work has been done in the area of polymeric prodrug systems, a lot of attention has been focused on the homing device section of such systems.[1] Antibodies — which can guide the drug specifically to desired sites — are the major attraction.

Polymeric prodrug systems are divided into two classes. In the first category, the link between the drug and polymer is broken extracellularly; in the second category, the drug is released intracellularly. The latter type of system also is commonly known as a *lysosomotropic device* because the substances are endocytosed and then degraded by the lysosomal enzymes or else by the acidic environment in the lysosomes.

Advantages of Polymeric Prodrugs

Polymeric prodrug systems offer several advantages. The attachment of a low-molecular weight drug to a macromolecule theoretically can alter the pharmaceutical, pharmacokinetic, and pharmacodynamic phases of drug action. A drug is exposed to many different enzymatic actions in vivo before it reaches the desired site. Yet because the drug in a polymeric system is "hidden" under a polymeric roof, the drug can avoid many degradation reactions and thus retain a more stable form. Furthermore, drugs with very little solubility are a problem to administer. The solubility of the polymeric–drug conjugate can be altered by selecting proper functional groups on the polymer backbone, and the increased solubility of the drug in water helps to minimize problems during the formulation process.

The cleavage of the drug from the polymer backbone is a function of the hydrolysis or enzymatic cleavage of the bond between the spacer molecule and the drug. By selecting a specific spacer molecule, prolonged release of a drug can be achieved. Macromolecules can improve a drug's bioavailability by reducing its gastric loss and metabolism. If the bond between the polymer and the drug is stable in gastric juice and is slowly hydrolyzed in the presence of a pancreatic enzyme in alkaline medium, the drug might not be released until it reaches the small intestine. For example, if a drug with a hydroxyl functional group is attached to the carboxylic group of an aromatic amino acid that is immobilized on a polymeric backbone, the resulting ester will be hydrolyzed specifically in the presence of α-chymotrypsin at an alkaline pH.[16] Because the molecular weight of these macromolecules is very high, they have a high renal threshold; consequently, the drug–polymer conjugate will not pass easily from circulation, and the blood circulation half-life of the drug will increase.

Targeted drug delivery systems provide either first order, second order, or third order targeting.[17] The greatest advantage of polymeric prodrugs is their ability to provide second and third order targeting — that is, their ability to release a drug at specific tissue sites or at preselected intracellular sites, respectively. A low-molecular weight drug can enter a cell by random diffusion. When the drug is attached to a polymer backbone that has a high molecular weight, the conjugate can only enter the cell by endocytosis, which can be a highly cell- and substrate-specific mechanism. Polymeric prodrugs can change a drug's distribution in different body tissues because of altered protein binding and different cell-specific interactions. One has to consider not only the distribution of polymeric prodrugs in the body but also the actual release of drug in different tissues. In vivo studies are needed in order to obtain this kind of information.

Selecting Macromolecule Carriers

The versatility of polymer chemistry allows many features that are essential for efficient drug delivery to be incorporated into the carrier macromolecule.[18] Among the factors to be considered when choosing a carrier system is the fact that the polymer must be able to bear functional groups to which the drug can be attached directly or by means of a spacer molecule. This bond must biodegrade at the site of action — extracellularly or intracellularly. In addition, there should be a sufficient number of drug molecules on the polymer backbone to allow for a sufficient concentration of the drug. Some drug conjugates are absorbed in the cells by receptor-mediated transport. In such cases, the carrier must retain its specificity for the receptors after it has conjugated with the drug.

The polymer must have a molecular weight that is great enough to prevent excretion — usually this means a weight greater than 40,000. Nevertheless, the molecular weight should be low enough to enable the conjugate to be captured pinocytically by target cells other than phagocytes.[19] It should be possible to incorporate homopolymer on the polymer backbone, which will facilitate the movement of the conjugate to the target cells and permit these cells to capture the conjugate by pinocytosis. Penetration into the cells can be enhanced by giving the polymeric carrier a positive charge or by raising its hydrophobicity. It has been shown that the incorporation of 20% tyramine residues into poly[α,β-*N*-(2-hydroxyethyl)-DL asparamide] greatly enhances the drug's rate of uptake by rat visceral yolk sacs. The incorporation of tyrosinamide residues into poly[*N*-(2-hydroxypropyl) methacrylamide] (HPMA) copolymer produced the same results.[20]

After the drug has been released, the carrier and its metabolic degradation products must be nonimmunogenic, nontoxic, noncarcinogenic, and should not alter the antigenicity of the transported drug. In addition, such degraded polymer products should not accumulate in the body. The carrier should meet reasonable criteria pertaining to pharmaceutical formulations, such as purity, solubility, and stability. The monomer–drug conjugate also should be polymerizable in systems where polymerization is carried out after the drug is combined with the monomers. In addition, the polymers must be readily available in ample supply and should not be very expensive.

Polymers as Drug Carriers

Polysaccharides. Polymers used in a polymeric drug delivery system are classified as either polysaccharides or synthetic polymers. The polysaccharides are either synthetic or endogenously available. Dextran and inulin are two polysaccharides that have been widely studied.[21,22] Sparer et al. proposed using glycosaminoglycans as drug carriers.[23] Cellulose and polyarabogalactan were also recently studied as possible drug carriers.[16]

Dextran is a synthetic polymer composed of linear chains of α-D-glucose molecules, 95% of which consist of 1,6-α-linked linear glucose units. The side chains of this polymer consist of 1,3-α-linked moieties. Dextran is biosynthesized by *Leuconostoc mesenteroides*.[22] The advantages of dextran as a drug carrier are its high degree of water solubility; its well-characterized, repetitive chemical structure; the availability of the polymer in

different molecular weight fractions ranging from 2×10^3 to 2×10^6; the polymer's low level of toxicity and pharmacological activity; and the protection from biodegradation provided by the substance for conjugated drugs or enzymes.[1] A question has been raised concerning the antigenicity of dextran, although available data demonstrates that immunological responses have been observed only for dextran fractions with molecular weights in excess of 100,000.[24]

In complexes of dextran and heavy metals, it has been found that the dextran–antimony complex is absorbed slowly from the injection site, and blood levels of the substance are maintained for several days. This complex has been used in the prophylaxis and treatment of an infection of *Leishmania donovani* in hamsters.[25] Dextran does not react with many drugs because of its lack of active sites; in such cases, it is activated using sodium periodate, succinic anhydride, 4-nitrophenyl chloroformate, carbomate, or cyanogen halides. Dextran–insulin was one of the first macromolecular compounds to be synthesized and studied.[26] The macromolecular derivatives of insulin showed a normal level of activity at much lower doses, and this activity lasted much longer than did that of native insulin. This phenomenon might be the result of a low degree of degradation of the polymer.

Dextran also is conjugated with different antibiotics, triazenes, and alpronolol.[27-29] Remon et al. investigated the pinocytic properties of dextran- and inulin-procainamide conjugates, which might ultimately be useful as sustained-release formulations. Also, the use of 2,4-dinitrophenol as an inhibitor of pinocytosis confirmed that the capture of macromolecular conjugate occurred by a pinocytic mechanism.[30] Sezaki and Hashida conjugated antibiotic mitomycin with dextran and the structure of this conjugate is shown in Figure 2.[31] If mitomycin C is attached by means of a cationic spacer molecule, cationic mitomycin C–dextran conjugate (MMCD) is formed. It was observed that cationic MMCD was adsorbed remarkably well on the surface of tumor cells by an electrostatic force, whereas mitomycin C (MMC) — or anionic MMCD — did not bind to the surface of such cells. In another study, it was found that anionic MMCD — which showed less growth-inhibiting activity in vitro against L1210 leukemia cells than did cationic MMCD — exhibited a greater degree of antitumor activity in vivo.[32]

Dextran with average molecular weights of approximately 10,000 (T-10), 70,000 (T-70), and 500,000 (T-500) were used in this study. *Increase in life-span* (ILS) is defined as follows:

$$\text{ILS} = (T/C^{-} - 1)$$

where

T = mean survival time of treated animals

C = mean survival time of control animals.

The value of ILS_{max} for MMCD anionic (T-500) was greater than that for MMCD cationic in intravenous administration because of the slow rate of drug release. Increased therapeutic indices were also observed for MMCD anionic.

The glycosaminoglycans (GAGs) are water-soluble, nontoxic, noninflammatory, nonimmunogenic, biodegradable, and endogenously available polymeric compounds. They bear a variety of functional groups, such as carboxyl, primary and secondary hydroxyl, and sulfate groups. Sparer et al. linked chondroitin sulfate, heparin, dermatan sulfate, hyaluronic acid (HA), and keratan sulfate to chloramphenicol, using an ester link to the alanine spacer group, which was itself an amide linked to the GAGs.[23] The rate of release of chloramphenicol from complexes of chondroitin sulfate (C_4 and C_6) was slower than the rate of release from hyaluronic acid complexes. HA, which has a higher molecular weight than chondroitin sulfate, might not be sulfated and therefore might be highly charged. Also, because more carboxyl groups on the HA formulation were left underivatized, the faster rate of hydrolysis might have been the result of an autocatalyzing effect. The amino acid cysteine also has been conjugated with GAGs, and this bond was found to break much more slowly than those linkages mentioned above.

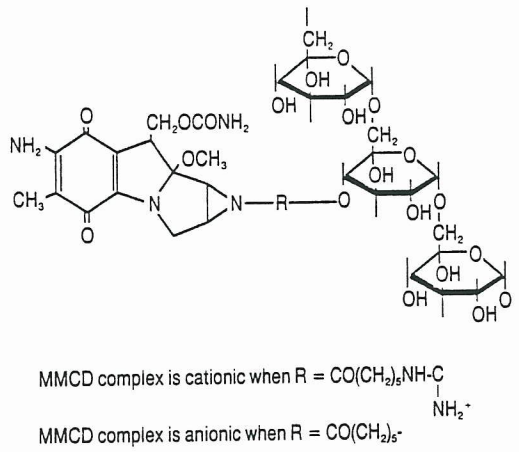

Figure 2: Chemical structure of dextran–mitomycin C conjugate (MMCD) with spacer molecules. In this example, the spacer group is cationic or anionic.

Lapicque and Dellacherie examined the synthesis of polymeric prodrugs of pholcodine that were prepared with various peptidic spacer arms and polysaccharide carriers.[16] The composition of the peptidic arms was intended to imitate that of known specific substrates of α-chymotrypsin. Two different polysaccharide carriers were used; the first was cellulose, which is water-insoluble, and the second was polyarabogalactan, which is water-soluble. The in vitro study showed that all the polymeric compounds were stable at a pH of 2.0 in the presence of pepsin and underwent only very slight hydrolysis of the ester function at a pH of 8.0, with and without trypsin. At the same pH level, hydrolysis was much faster in the presence of α-chymotrypsin. The low-molecular weight prodrugs — without a polysaccharide backbone — which had the same peptidic spacer arms released the drug 10^3 to 10^4 times faster than did the polymeric prodrugs. In addition, the prodrug that contained a water-soluble polymeric carrier had a release rate 10 times faster than that of a prodrug with a water-insoluble polymeric carrier.

In all this research, no one has considered the three-dimensional structure of polysaccharide macromolecules. Consideration of the bonding mechanism and geometry of these polysaccharides might be helpful in understanding the mechanism of chemical synthesis of prodrugs and the enzymatic hydrolysis of polymeric prodrugs by which drug is released. Atkins compared the structure of polysaccharides with that of their protein counterparts.[33] As with proteins, polysaccharides have α-helix, β-sheet, and helical structures. Cellulose has glucose units linked diequatorially through carbon atoms at ring positions 1 and 4 (1e-4e) and it exhibits features that are closely similar to the classic β–pleated sheet structures. The starch molecule has amylose units linked through carbon atoms in ring positions 1 and 4 (1a-4e); the polysaccharide chain traces out a hol-

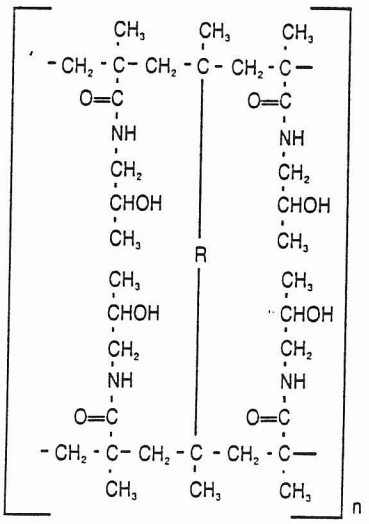

Figure 3: Chemical structure of the cross-linked HPMA copolymer. R = *drug molecule or oligopeptide.*

low tube-like structure, which is stabilized by interchain hydrogen bonds between successive turns of the helix. This structure mimics the commonly occurring α-helix found in proteins. The curdlan, an extracellular microbial polysaccharide, has glucose units linked diequatorially through atoms at ring positions 1 and 3 (1e-3e). The molecule has a three-strand, solid rope-like structure with individual chains that fit together neatly.

Understanding the structure of polysaccharides will help to explain the disposition of functional groups to which drugs can be conjugated. Because the formation of prodrug might change the geometry of a polysaccharide — thus changing its position — the stereochemistry of the polymeric prodrug system must be studied in detail.

Synthetic polymers. Synthetic polymers are widely used as drug carriers because the properties of these molecules can be modified by varying their structure. Polylysin, polyglutamic acid, HPMA, and polyphosphazene are some of the most commonly used polymers.

Poly(L-lysine) (PLL) is a homopolymer of repeating L-lysine units. Because lysine is a basic amino acid at a physiological pH, the polymer bears a positive charge. Tumor cells readily take up PLL by pinocytic action, and the conjugation of PLL to albumin and horseradish peroxidase markedly enhanced the cellular uptake of both proteins. PLL itself showed some antibacterial and antiviral activities and was found to be toxic to animals at elevated doses or in high-molecular weight forms.[34]

Prodrugs of methotrexate with PLL were studied extensively, and a PLL derivative of methotrexate was found to be more active than a poly(D-lysine) derivative of methotrexate. The PLL-methotrexate is a lysosomotropic drug-carrier system, in which lysosomal enzymes release methotrexate in the cells.[35] Friend and Pangburn pointed out that the extrapolation of results obtained in vitro to events expected to occur in vivo can be misleading.[1] Mechanisms for the distribution and metabolism of polymeric drugs were generally missing in most of the in vitro tests, and poor correlations between in vitro and in vivo results were observed. The authors stressed the need to study the distribution of polymeric drug systems using labeled polymers or drugs.

HPMA is another widely studied lysosomotropic drug-carrier system.[36-39] The structure of HPMA is shown in Figure 3. In this structure, *R* can be a drug or a cross-linked oligopeptide sequence. By choosing the proper oligopeptide, soluble HPMA copolymer can be synthesized.[36] Although HPMA is not biodegradable, the oligopeptide cross-links have some degree of biodegradability mainly because of the lysosomal enzymes. The proper sequence of amino acids will make the polymer susceptible to chymotrypsin, trypsin, and papain.[40-42] Kopecek found that the bond between the terminal amino acid and the *p*-nitroaniline was cleaved in all the cases rather than the oligopeptide linkages.[20] Carbohydrate residues, glycoproteins, and antibodies were used as specific targeting moieties.[1] The use of antibodies for targeting has enormous potential, but the extent of direct covalent-binding of drug molecules was found to be limited by the progressive loss of activity or solubility or both.[20,38,43] The molecular weight of HPMA is an important criteria. Although the rate of uptake of HPMA by the yolk sac decreased markedly when molecular weight increased, molecular weight had no effect on the extent of intracellular degradation of HPMA.[36,44]

In a recent experiment, Cartlidge et al. studied the blood clearance and body distribution of different molecular weight fractions of HPMA in rats.[44] For all the routes of administration, HPMA was found in the bloodstream before being eliminated in urine and feces. The copolymers were found to be stable in plasma but were degradable by lysosomal enzymes. In another study, the same authors synthesized HPMA copolymers with an oligopeptide side chain (Gly-Gly) that terminated in ester-linked *p*-nitrophenol and was cross-linked with di(phenylalanyl) hexamethylene diamine.[45] The authors also synthesized a sample with galactosamine residues bound to the cross-linked polymer by means of the Gly-Gly side chain. Following intravenous administration, galactosamine that contained HPMA copolymer was cleared from the circulation more rapidly than was the unsubstituted polymer. The most important observation was that the drug was targeted to the liver after intraperitoneal and subcutaneous administration.

McCormick et al. observed that the cationic HPMA copolymer was associated progressively with rat visceral yolk sacs during a 5-h incubation period.[46] Inhibitor studies involving low temperatures or the addition of 2,4-dinitrophenol indicated that this association was not primarily a result of pinocytic capture of the polymer but rather was caused by adsorption onto the cell surface. The release of cationic HPMA after the cleavage of drug from the tissue and clearance from the bloodstream also was rapid. The cell surface is known to display a net negative charge, and the authors predicted that electrostatic attraction would occur between the cell and the trimethyl ammonium group of the HPMA copolymer. HPMA copolymers that contained oligopeptide sequences terminated in various functional groups and had no prominent effect on the porcine complement system in vitro; nevertheless, at very high doses, inhibition of both pathways of the complement system was observed.[47]

Recently, Grolleman et al. prepared a polymer prodrug of naproxen with polyphosphazene, using a spacer molecule.[48,49] The authors made an implantable bioerodible system and studied its release kinetics. In the in vivo experiments in rats after implantation of the polymeric device, levels of naproxen in the blood and urine were measured; after an initially high rate of release, a very slow release rate of naproxen was observed. Gros et al. synthesized a polymeric conjugate of poly(glutamic acid) and *p*-phenylenediamine, using immunoglobulin as a homing de-

vice.[50] The *p*-phenylenediamine that was released from the polymer prodrug showed a greater level of antitumor activity, and the results also indicated that the routes of inoculation and administration of the conjugate were very important factors.

The recent increase in the use of new synthetic polymers makes it necessary to screen all polymer materials thoroughly. Darby suggested that polymers should be screened in three steps: chemical screening, biological tests, and protocol tests.[51] The polymer's immunogenicity, hemolytic activity, pyrogenicity, osmotic properties, and its interaction with the components of plasma must be studied before the polymer can be used in drug delivery systems. For example, even endogenously found polymers such as chondroitin sulfate or HA might show toxic effects after prolonged use at very high doses.

Conclusion

Until recently, a polymeric prodrug system has been used only in intravenous administration; but, as in case of phosphazene, such a system can now be used as a bioerodible implant. Localized effects, as in the case of gastrointestinal delivery,[16] can be used effectively, and there is no doubt about the usefulness of this system. To design polymeric prodrugs, one must apply knowledge concerning tumor cells, bacterial cells, and normal human cells, such as their biochemical nature and various receptor sites. Polymeric prodrugs have tremendous potential for use as a site-specific delivery system, and the variety of their applications have yet to be explored. In particular, in vivo studies must be carried out in order to demonstrate the prolonged release rate or site specificity of this system.

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