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Review

Macromolecular Carrier Systems for Targeted Drug Delivery: Pharmacokinetic Considerations on Biodistribution

Yoshinobu Takakura[1] and Mitsuru Hashida[1,2]

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This review article describes the current status and future perspectives of site-specific drug delivery by means of macromolecular carrier systems. Basic aspects and recent advances of targeted delivery of 1) conventional drugs, 2) protein drugs, and 3) gene medicines including antisense oligonucleotides and plasmid DNA, are reviewed from a pharmacokintic perspective. Successful in vivo application of macromolecular carrier systems requires pharmacokinetic considerations at whole body, organ, cellular and subcellular levels. The integration of simultaneous research progress in the multidisciplinary fields such as biochemistry, cell and molecular biology, pharmacology, and pharmacokinetics will accelerate the emergence of marketed drugs with macromolecular carrier systems.

KEY WORDS: macromolecular carrier; pharmacokinetics; targeting; protein drugs; gene medicines.

INTRODUCTION

For effective therapy with medication, it is necessary to deliver therapeutic agents selectively to their target sites, since most drugs are associated with both beneficial effects and unfavorable actions. This selectivity is best for antitumor drugs because of their extreme cytotoxicity. In general, the lack of selectivity of most conventional drugs is closely related to their pharmacokinetic properties. The in vivo fate of a drug given by a particular administration route is determined by both the physicochemical properties of the drug and anatomical and physiological characteristics of the body. Most conventional drugs diffuse freely throughout the body and show relatively even tissue distribution due to their low molecular weight.

Among the various strategies for site-specific drug delivery, that of macromolecular carriers can be a formidable tool because of their diversity in physicochemical and biological properties and functions (1–3). The rationale for a macromolecular carrier approach in site-specific drug delivery lies in the altered disposition of a carrier-conjugated drug in the body, which is largely dictated by the properties of the carrier and accordingly differs from that of the free drug administered by the same route. In this context, it is important to understand the pharmacokinetics of macromolecules in relation to their physicochemical and biological characteristics.

The progress in biotechnology, such as being able to synthesize DNA constructs containing genes of interest, has effected dramatic changes in therapeutic modalities. While only "xenobiotics" have been used mostly as medicines in conventional drug therapy, the use of endogenous macromolecules and related substances as therapeutic moieties have become increasingly common. The first generation of therapeutic recombinant proteins has already been clinically applied. Efforts have also been made to develop more effective delivery systems for improving the pharmacokinetic properties of proteins drugs and to render proteins more realistic as drug candidates in therapeutics (4). Macromolecular carriers may be useful for some of these protein drugs.

Furthermore, recombinant DNA itself has been used like a "drug" in the novel therapeutic methodology of gene therapy, in which a variety of diseases may be treated by transferring genetic material into specific cells of a patient (5). On the other hand, the inhibition of gene expression using antisense oligonucleotides, which are relatively small synthetic DNA fragments designed to hybridize specific mRNA sequences in target cells, can be considered as a novel type of chemotherapy (6). Macromolecular carriers have been used for the targeted delivery of these DNA drugs "gene medicines", for therapy at the level of gene expression.

This article reviews the current status and future prospects of targeted drug delivery with macromolecular carrier systems. For the rational design of such targeted drug delivery systems aiming at controlled biodistribution, pharmacokinetic aspects are important (7). In this review, the site-specific delivery of conventional drugs, protein drugs and gene medicines by the use of macromolecular carriers is discussed based on pharmacokinetic considerations at the whole body, organ, and cellular levels.

Design of Drug-Macromolecule Complexes

A variety of natural and synthetic macromolecules have been used as drug carriers. The criteria for choice of macromo-

[1] Department of Drug Delivery Research, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606-01, Japan.
[2] To whom correspondence should be addressed.

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lecular carriers can be summarized as follows. The carrier should (a) be biocompatible, (b) lack intrinsic toxicity and antigenicity, (c) not accumulate in the body, (d) have adequate functional groups for chemical fixation, (e) retain the original specificity for target, and (f) maintain the original activity of the delivered drug until it reaches the site of action. The design of drug-macromolecule complexes can be classified into three categories depending on the drug type.

Macromolecular Prodrugs

For most low molecular weight drugs, drug-macromolecular carrier conjugates are classified as macromolecular prodrugs (1). Although some polymer-bound antitumor drugs exhibit activities in the conjugated form without liberating free drugs (8), most conjugates exhibit pharmacological efficacy after conversion to the parent compounds by virtue of an enzyme or chemical lability, or both, before or after reaching the site of action in the body.

In designing macromolecular prodrugs, the pharmacologically active component should (a) show enough activity at relatively low doses to decrease the load of a carrier macromolecule (cytotoxic agents are often used for this reason), (b) be chemically stable in the conjugated form until released, and (c) have adequate functional groups in its molecular structure for chemical fixation (1). In addition, the chemical and/or biological stability of the linkage between the drug and the macromolecular carrier should be considered since their pharmacological effectiveness requires the release of the free drug from the conjugate. Generally, drugs are coupled directly to functional groups in a macromolecular carrier or to spacers introduced to a macromolecular backbone via covalent bonds, using various cross-linking agents. Functional groups in the drug and the carrier molecule used for chemical coupling include amino, carboxyl, hydroxyl groups, and free thiol groups. Most of the reactions are performed in aqueous media under mild conditions to avoid denaturation of the parent drug and the macromolecular carrier. These linkages between the drug and the carrier must be designed to be cleavable at an appropriate rate to act as prodrugs. When the linkage is to be cleaved by enzymatic reaction(s), animal species differences in the type and activity of the enzyme(s) should be considered.

Protein-macromolecule Conjugates

Macromolecular carriers have been used to chemically modify protein drugs to improve their pharmaceutical, pharmacokinetic and immunological properties. In general, protein drugs are chemically conjugated to macromolecular modifiers and the conjugates, hybrids of two macromolecules, can have unique biological activities unlike those of macromolecular prodrugs. In this case, proteins must have appropriate functional groups, solubility and stability for chemical modification and conjugation. The molecular weight of modifiers and extent of conjugation should be selected to maintain the biological activities of the protein.

Occasionally, conjugation with macromolecular carriers attenuates the biological activities of protein drugs by means of an intrinsic conformational change and/or restricted accessibility to target molecules due to steric hindrance. Enzymes have been most widely used since their substrates, which are usually highly diffusivable small molecules, should undergo relatively effective reactions catalyzed by the enzyme even after modification (9). Although steric hindrance should be considered especially for protein drugs that require receptor binding to exert their therapeutic action, several conjugated cytokines can maintain their activities (10).

In addition to macromolecular conjugation, direct chemical modification with small functional moieties that alter the physicochemical and biological properties of proteins would be useful. These moieties include positively or negatively charged groups, lipophilic groups and sugars. The basic concept similar to macromolecular conjugation can be applied to the design of protein drugs that will be directly modified with these small molecules.

Gene Medicines Complexed with Macromolecular Carriers

For successful therapy with gene medicines involving recombinant plasmid DNA and antisense oligonucleotides, it is necessary to develop carrier systems that deliver these materials to the target intracellular site. In general, gene medicines have substantial problems as polyanionic DNA molecules. These include susceptibility to degradation by nucleases and low membrane permeability. The introduction of an appropriate macromolecular counterpart as a carrier of gene medicines would be one useful way to circumvent these problems.

Macromolecular carriers have been covalently attached to oligonucleotides (11). However, the use of non-covalent electrostatic interaction between DNA and polycations is more common (12). Current bifunctional macromolecular conjugates consist of two components, a target recognizable macromolecule and a polycation. The former portion would direct the conjugate to its target cells and the polycationic region can bind DNA based on electrostatic interaction and neutralize anionic charges. Small target recognition elements such as sugars have been introduced directly to polycations. Various ligands that can bind to receptors on the specific cell types such as glycoproteins, transferrin, and insulin, and monoclonal antibodies capable of recognizing cellular epitopes have been used as the targeting component. On the other hand, poly-L-lysine is the most common polycation for the hybrid carrier.

Many factors should be considered in designing a carrier system. These include (a) the choice of targeting moiety, (b) the molecular weight of poly-L-lysine (or other polycation), (c) the coupling ratio of these components, (d) mixing molar ratio of the carrier and DNA, (e) conditions such as ionic strength for mixing of the carrier and DNA (for a review, see ref. 12). Apparently, these factors are dependent on the type and size of the DNA.

Targeting and Macromolecular Carriers

Site-specific drug delivery is broadly categorized as passive and active targeting (13). "Passive targeting" refers to the exploitation of the natural (passive) disposition profiles of a drug carrier, which is passively determined by its physicochemical properties relative to the anatomical and physiological characteristics of the body as will be discussed later. "Active targeting" refers to the alterations of the natural disposition of a drug or carrier, directing it to specific cells, tissues, or organs. Ligands or monoclonal antibodies which can bind specifically to the surface of target cells, are used for this purpose. Active targeting seems to be much more attractive than passive tar-

geting; consequently, many efforts have focused on the development of sophisticated macromolecular carriers with a specific recognition potential.

The distribution and elimination of the macromolecular carriers are mainly dictated by their physicochemical properties such as molecular size, electric charge, hydrophilic/lipophilic balance, etc. Even if molecules that can function as a target-recognizing device when facing the target are an integral of the conjugate, the physicochemical properties still play an important role in deciding the localization of the complex in the target tissue. Therefore, a precise understanding of the general relationship between the physicochemical properties of macromolecular carriers and their disposition behavior is a major prerequisite for not only passive, but also active targeting.

The stage or level of targeting is another important aspect of drug delivery. Widder et al. (14) have defined three distinct stages: first-, second-, and third-order targeting. First-order targeting refers to distribution restricted to the target organ or tissue; second-order targeting refers to selective direction to the target cells; third-order targeting refers to delivery to selected intracellular sites. Although this classical definition for drug targeting was originally given for antitumor agents, this concept will be useful to construct the targeting strategy for any drugs using macromolecular carriers. It should be possible to control the pharmacokinetics of drugs of interest at the organ, cellular, and subcellular levels by selecting the optimal macromolecular carriers.

Fate of Macromolecules in the Body

Anatomical and Physiological Considerations

The capillary endothelium comprises a dynamic interface adapted for exchange of endogenous and exogenous substances between the blood and the interstitial fluids. The basic structural features of capillaries are diverse among organs. Based primarily on modulations in the fine structure and continuity of the endothelium and its basal lamina, there are three main types of blood capillaries: continuous, fenestrated, and discontinuous (15).

The continuous capillaries are the most widely distributed in mammalian tissues and are found in skeletal, cardiac, and smooth muscles, as well as in lung, skin, subcutaneous tissues, serous and mucous membranes. The transport pathways important in macromolecule exchange in these capillaries include pinocytotic vesicles, intercellular junctions, and transendothelial channels. Physiological estimates of transport pathways for macromolecules have revealed small pores with radii of 6.7–8.0 nm and large pores (20–28 nm). As serum albumin with an effective diameter of 7.2 nm (Mw = 67,000) can barely pass through the pores, the substantial transport of macromolecules across the continuous capillaries is negligible. The brain capillaries have the most outstanding feature as a blood-brain barrier for highly restricted macromolecular transport (16).

Fenestrated capillaries are generally found in the intestinal mucosa, the endocrine and exocrine glands, and the glomerular and peritubular capillaries of the kidney. Macromolecules can move across the endothelial barrier in the same fashion as described for continuous capillaries. In addition, macromolecules can also move through the fenestrae, circular openings with radii of 20–30 nm and which are commonly closed by a diaphragm. In glomerular capillaries, the fenestraeare devoid of diaphragms and macromolecules can cross the fenestrated capillaries easily. However, the continuous basement membranes function primarily as a size and charge selective barrier for glomerular filtration of macromolecules. The shape, flexibility, and deformability of macromolecules are also important factors here (15).

The distribution of the discontinuous type of capillary wall is more limited than that of the other types; they are found only in the liver, spleen, and bone marrow. These capillaries are characterized by endothelial gaps, intercellular junctions with diameters ranging between 100 and 1,000 nm and an absence of a basement membrane. In these capillaries, the openings are extremely large and do not restrict the passage of macromolecules between plasma and interstitium. The capillary wall of the liver is characterized by fenestrae of about 100 nm with a porosity of 6–8% (17).

In addition to these features under normal conditions, capillary permeability to macromolecules is enhanced in pathophysiological states such as cancer and inflammation (18). Thus, the fate of administered macromolecular carriers is determined based on their physicochemical properties such as molecular weight and electric charge in relation to the anatomical and physiological features described above. Blood-born macromolecular carriers would distribute to the tissues, according to differences in organs between normal and diseased states. Therefore, passive first-order targeting after systemic administration will be simply achieved by using appropriate macromolecular carriers. Although intralesional targeting or lymphatic targeting can also be achieved by macromolecular carriers after local administration (19), targeted delivery after systemic intravascular administration is focused in this review.

General Pharmacokinetics of Macromolecules

Immediately after intravenous injection, the distribution of macromolecules is basically restricted to the intravascular space due to low capillary permeability in most organs as described earlier. The kidneys play an important role in the disposition of macromolecules circulating in the vasculature. Macromolecules with a molecular weight of less than 50,000 (approximately 6 nm in diameter) are susceptible to glomerular filtration and are excreted into the urine. Since the glomerular capillary walls also function as a charge selective barrier having negative charges, positively charged macromolecules show higher glomerular permeation than anionic macromolecules of similar molecular weights. For larger macromolecules that escape sieving through the glomerulus, the liver plays an important role. In contrast to most other organs in which the capillary presents a substantial barrier between the vascular and interstitial spaces, the structure of the discontinuous endothelium of the liver brings circulating macromolecules in the blood into free contact with the surface of parenchymal cells. Due to this anatomical feature which offers a wide surface area for electrostatic interaction, cationic macromolecules are highly distributed to the liver based on adsorption on the negatively charged cell surface (20). On the other hand, polyanions are taken up by liver non-parenchymal cells by scavenger receptor-mediated endocytosis (21,22).

The pharmacokinetic properties of macromolecules can be analyzed based on a simple physiological pharmacokinetic

model. Assuming unidirectional organ uptake, the general disposition characteristics of macromolecules can be characterized using organ uptake clearance (CLorg) as an index (23). Pharmacokinetic studies based on this clearance concept have quantified general disposition characteristics of macromolecules, such as polysaccharides, proteins, and DNA (21–26). In Figures 1–3, the hepatic uptake and urinary excretion clearance which essentially decides the disposition of macromolecules at the whole body level are plotted in a logarithmic scale for a variety of macromolecules. Figure 1 shows that urinary excretion is highly dependent on molecular weight. On the other hand, charge affects hepatic uptake as well as renal excretion (Figures 2 and 3). Positively charged macromolecules have large hepatic uptake and renal excretion clearance. In particular, cationic proteins are characterized by remarkable renal accumulation, which is due to both tubular reabsorption and uptake from the peritubular capillary side (27). Hepatic uptake clearance is larger for highly anionic macromolecules such as DNA and succinylated proteins (Figure 3). The relationship between the general disposition characteristics of macromolecules in the kidney and liver and their physicochemical properties is thus demonstrated.

Fate of Macromolecules in the Cells

Except for protein drugs that usually show biological actions upon binding to cell surface receptors, the site of action of most agents with macromolecular carrier systems is usually intracellular, for example, nuclei for antitumor drugs and genes. Although macromolecular prodrugs of mitomycin C (3) and low molecular weight prodrugs in combination with antibody-enzyme conjugates (28) can exhibit cytotoxicity by releasing free drug in the extracellular space, the therapeutic efficacy of

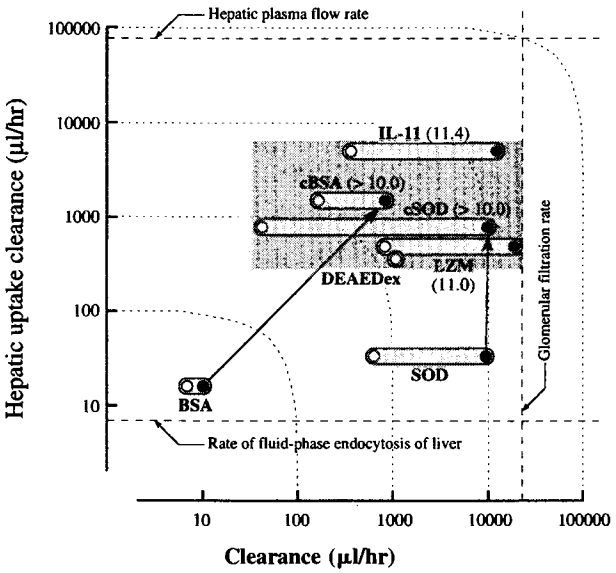


Fig. 2. Hepatic uptake and urinary excretion clearances of cationic macromolecules in mice after intravenous injection. SOD; recombinant human superoxide dismutase; cSOD; cationized SOD; BSA, bovine serum albumin; cBSA, cationized BSA; LZM, lysozyme; IL-11, recombinant human interleukin 11; DEAEDex, diethylaminoethyl-dextran. Numbers in parentheses are isoelectric points. Open circles represent urinary excretion clearance and closed circles represent the total of urinary excretion and kidney uptake clearance. Shaded bars between open and closed circles correspond to accumulation in the kidney. Arrows indicate the effect of cationization of the original macromolecules.

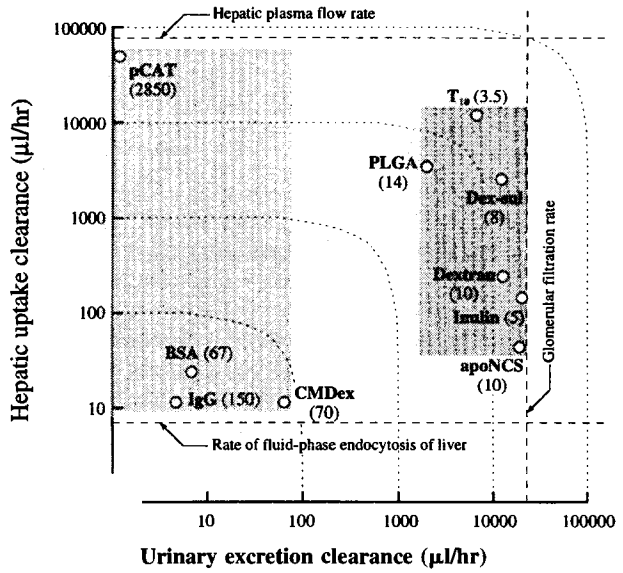


Fig. 1. Hepatic uptake and urinary excretion clearances of macromolecules in mice after intravenous injection. Effect of molecular weight. IgG, immunoglobulin G; BSA, bovine serum albumin; apoNCS, apoprotein of neocarzinostatin; CMDex, carboxymethyl-dextran; Dex-sul, dextran sulfate; PLGA, poly-L-glutamic acid; pCAT, plasmid DNA encoding chloramphenicol acetyltransferase; T_{10}, thymidine decamer. Numbers in parentheses are approximate molecular weights in kilo.

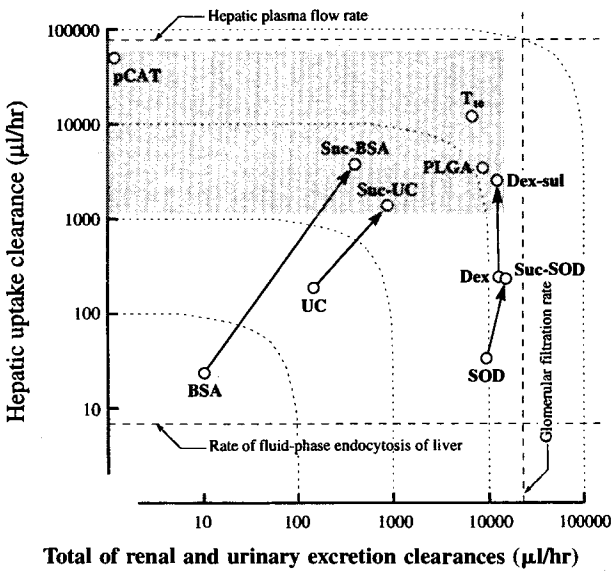


Fig. 3. Hepatic uptake and urinary excretion clearances of anionic macromolecules in mice after intravenous injection. Suc-SOD; succinylated SOD; Suc-BSA, succinylated BSA; Suc-UC, succinylated uricase; Dex-sul, dextran sulfate; PLGA, poly-L-glutamic acid; pCAT, plasmid DNA encoding chloramphenicol acetyltransferase; T_{10}, thymidine decamer. Succinylated proteins had isoelectric points below 4.0. Arrows indicate the effect of anionization of the original macromolecules.

most of drug-carrier complexes greatly depends on cellular uptake and intracellular trafficking as well as the intracellular release of active drug component.

Cellular Uptake

Since macromolecules normally cannot enter cells by passive diffusion across the plasma membrane, the general mechanism whereby they pass the cell membrane is endocytosis. This is a widespread process of cell surface invagination and subsequent internalization of plasma membrane as vacuoles and it is associated with the transport of extracellular solutes into the cell. A macromolecule dissolved in the extracellular fluid can enter a cell with medium but at relatively slow rate. This process is called "fluid-phase endocytosis". Macromolecular carriers without any specific affinity to target cells are considered to be endocytosed by this mechanism. In "adsorptive endocytosis", macromolecules bound to the plasma membrane are internalized at rates usually faster than those for fluid-phase endocytosis. Cells may endocytose cationic macromolecules by this process following adsorption on the anionic plasma membrane via the electrostatic interaction. Macromolecular carriers for active targeting, such as glycoproteins, hormones, and lectins are rapidly and effectively internalized via receptor-mediated endocytosis which proceeds mostly at clathrin-coated pits. The rate and extent of endocytosis of drug-macromolecular carrier complexes are primary factors afecting their therapeutic efficacy.

Intracellular Pharmacokinetics

In endocytosis, the coat is shed and uncoated endosomes are formed after the formation of clathrin-coated vesicles. These vesicles are relatively small (100 nm in diameter), and the size of the ligand cannot exceed them. This size restriction is important especially for the receptor-mediated targeting of genes that become fairly large when complexed with polycation-ligand conjugates (12). Although there are several possible fates for ligands using the coated pit pathway following internalization, fusion with lysosomes enriched with hydrolytic enzymes yields digested vacuole and the complexes are degraded. This pathway is favorable to macromolecular conjugates of small antitumor agents such as anthracyclines. These macromolecular prodrugs are designed to conform to the concept, "lysosomotropic" delivery (29). Here, the free drug is regenerated within the lysosome following digestion of the carrier and it effectively reaches the targeted intracellular compartment such as the nucleus due to its high diffusivity and stability in the milieu.

On the other hand, processes such as escape from the endosomal and/or lysosomal degradative pathways and successive translocation to the cytosol or nucleus should be considered for genes and antisense oligonucleotides. Resistance to degradation is much more important throughout these processes. However, little is known about the intracellular fate of macromolecular carrier systems. With regard to nuclear transport, direct entry into the nucleus occurs via nuclear pores which are reportedly smaller than 40 nm (11) in diameter. This implies that macromolecular carrier systems targeted to the nucleus should be smaller than the pores.

Passive Targeting

Passive Tumor Targeting

Tumor tissues are characterized by increased interstitial pressure which may retard the extravasation of macromolecules. On the other hand, large vascular permeability and high interstitial diffusivity of macromolecules seem to facilitate their migration to tumor tissues. In addition, a lack of functional lymphatic drainage will result in the accumulation of macromolecules by a "passive" mechanism. Thus, these anatomical and physiological characteristics of solid tumors would provide a reliable rationale for the use of macromolecular carrier systems in tumor targeting (3,18,30). Capillary vessels in a human tumor inoculated into SCID mice are permeable even to liposomes of up to 400 nm in diameter (31).

On the basis of the clearance concept, targeting efficacy, the total amount of the macromolecule that reached the target site over infinite time, is estimated as a ratio of CLorg at the target site and the total body clearance (CLtotal). Under these conditions, it is apparent that macromolecules with a high CLorg at the target site but with a small CLtotal accumulate at high levels in the target site.

Table I summarizes the pharmacokinetic parameters including the tumor uptake rate (clearance) of macromolecules determined in a variety of murine tumor models (23,24,32,33). Although a direct comparison of these values would be problematic due to differences in tumor types and other conditions, the absolute values of tumor uptake of monoclonal anti-tumor antibodies do not substantially differ. Although these values variy depending on the physicochemical properties of macromolecules, the results demonstrate that the changes are significantly smaller compared with those in CLtotal, which are also affected by physicochemical properties. Therefore, macromolecular prodrugs of a suitable molecular size (Mw $>$ 70,000) and a slightly anionic nature will have prolonged retention in the plasma circulation followed by large accumulation in the target, such as a tumor. This notion has been validated by the fact that an anionic macromolecular prodrug of mitomycin C (mitomycin C-dextran conjugate) with a molecular weight of 70,000, markedly accumulated and subsequently inhibited the growth of solid tumors subcutaneously inoculated into mice (23). Furthermore, in an active targeting attempt, a mitomycin C-monoclonal antibody A7 conjugate using anionic dextran as an intermediate with a low CLtotal, remarkably accumulated in a targeted human colorectal tumor implanted into nude mice (Table I). The intravenous administration of the conjugate inhibits the growth of the target tumor SW1116 inoculated into nude mice (32). Thus, these studies have demonstrated the simple but important principle that a macromolecular carrier system with an extended plasma circulation can result in a large accumulation in the tumor in both passive and active targeting.

Polycation-mediated Targeting

The electrostatic interaction of polycations with the cell surface is essentially non-specific. However, the liver plays a significant role in the overall clearance of large cationic macromolecules from the circulation because of its unique capillary architecture as discussed earlier. Thus, straightforward first-order passive targeting to the liver can be attained by

Table I. Pharmacokinetic Parameters for Various Macromolecules After I.V. Injection in Tumor Bearing Mice

Compound	Molecular weight	Charge	AUC (% of dose hr/ml)	CL total (μl/hr)	CL muscle (μl/hr/g)	CL tumor (μl/hr/g)	Total Tumor[a] Accumulation (% of dose)	Tumor cells	Reference
Dextran (T-10)	9,900	neutral	6.6	15195	65.4	236	0.48		
Dextran (T-70)	64,400	neutral	146	685	2.5	23.9	1.05	Sarcoma	(24)
DEAE-Dextran (T-70)	64,400	+	51.1	1957	6.7	59.1	0.9	180	
CM-Dextran (T-70)	64,400	−	1009	99.1	1.1	15.5	4.68		
NCS	10,700	−	3.8	26175	26.6	132	0.24		
BSA	70,000	−	764	131	1.8	14.2	3.24	Sarcoma	(24)
cBSA	70,000	+	48.2	2075	5.4	36.9	0.39	180	
IgG	150,000	−	1623	61.6	1.2	12.8	6.24		
MMCDan (T-10)	10,000	−	98.9	1012	2.8	23.4	0.69		
MMCDan (T-70)	70,000	−	431	232	1.0	13.6	1.77	Sarcoma	(23)
MMCDan (T-500)	500,000	−	741	135	0.5	8.9	1.98	180	
MMCDcat (T-70)	70,000	+	15.1	6623	7.3	27.1	0.15		
MAb (Specific)-MMCDan	220,000	−	526	190	2.0	59.8	9.45	SW 1116	(32)
MAb (Nonspecific)-MMCDan	220,000	−	236	424	3.2	23.8	1.68	Sarcoma 180	
Diphtheria toxin (DT)	60,000		171	584	11.4	17.4	0.89		
Mutant of DT (mDT)	60,000		192	521	10.8	21.6	1.24	TE671	(33)
MAb (Specific)-mDT	210,000		744	134	4.8	7.8	1.74		
MAb (Nonspecific)-mDT	210,000		153	652	7.8	15	0.69		

[a] Calculated at infiite time after i.v. injection by assuming the tumor weight to be 0.3 g. DEAE-Dextran, diethylaminoethyldextran; CM-Dextran, carboxylmethyldextran; NCS, neocarzinostatin; BSA, bovine serum albumin; cBSA, cationized BSA; IgG, mouse immunoglobulin G; MMCDan, mitomycin C-dextran conjugates with anionic charge; MMCDcat; mitomycin C-dextran conjugates with cationic charge; DT, Diphteria toxin (binding); mDT, mutant of DT (nonbinding); MAb, monoclonal antibody; TE671, human rhabdomyosarcoma; SW1116, human colon cancer.

using cationic macromolecular carriers. The hepatic targeting of cationic conjugate of mitomycin C (23), and superoxide dismutase with dextran derivatives (34) has been reported.

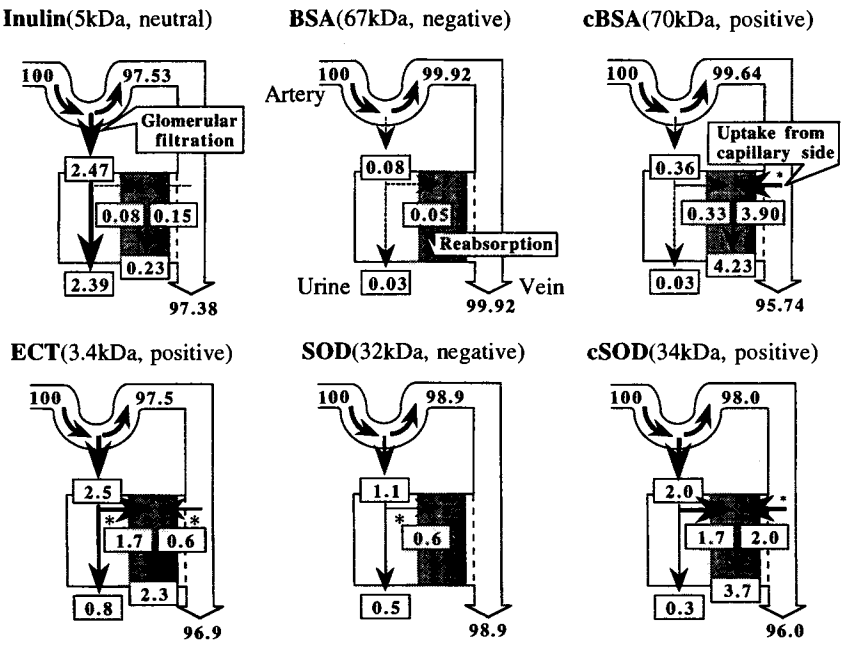
Cationic proteins, such as cationized albumin, cationized immunoglobulin G, histone, CD4, and avidin, can be transported across the brain capillary endothelium by adsorptive-mediated transcytosis (35). Although the absolute targeting efficiency (amount) is not very high due to the predominant total body clearance, the cationic macromolecular carrier systems may be useful for delivering drugs to the brain across the endothelium comprising the blood-brain barrier. This approach has been applied to peptides and oligonucleotides.

The kidney is also an important organ for the uptake of cationic macromolecules as previously discussed. The renal handling of model macromolecules including polycations has been evaluated using isolated perfused rat kidneys by which the renal disposition processes of glomerular filtration, tubular reabsorption, and uptake from capillary side, can be measured (27). The results shown in Figure 4 suggest that large cationic macromolecules can be used for renal targeting from the peritubular side while smaller ones are efficiently delivered from both luminal and capillary sides. Based on the results, successful renal targeting and improved therapeutic potency of superoxide dismutase (SOD) was obtained by direct cationization (36). On the other hand, Franssen et al. (37) have developed macromolecular prodrugs for renal targeting using the cationic low molecular weight protein, lysozyme (Mw 14400) as a carrier. Here, the drug-macromolecule conjugates were designed to release active free drug by lysosomal digestion after endocytosis (reabsorption) by proximal tubular epithelial cells following glomerular filtration.

Persistence of Protein Drugs in Blood Circulation

Clinical application of protein drugs especially recombinant proteins is often limited by their very short biological half lives after intravenous injection. Care must be taken in changing the pharmacokinetic profiles of protein drugs since their biological activities are sometimes not simply related to the pharmacokinetics (38). In addition, a variety of factors affect the pharmacokinetics of protein drugs. For example, down regulation of receptors is a critical issue for protein drugs which are taken up by receptor-mediated endocytosis as discussed by Sugiyama and Hanano (39). However, the prolonged blood circulation of some protein drugs may bring about a better therapeutic effect.

Although various factors, such as proteolytic degradation, reticuloendothelial uptake, and receptor-mediated clearance are involved in the rapid elimination, one major problem inherent in for these proteins is susceptibility to glomerular filtration. Most therapeutic cytokines such as interferons, interleukins, and tumor necrosis factors have molecular weights ranging from 10,000 to 30,000. Conjugation with macromolecular carriers is a simple and effective way to reduce their glomerular filtration and subsequently prolong their blood circulation. In a sense, this approach may be defined as passive targeting to the intravascular spaces. Increased retention in the circulation of a protein will be useful if the target cells or molecules are in the intravascular space. Alternatively, the enhanced blood circulation may be advantageous for passive targeting to the tissue with the permeability of the modified protein being better than the original. The biocompatible macromolecules albumin, dex-



Values in each process are expressed as the percent of dose
***Nonlinear process**

Fig. 4. Renal disposition profiles of macromolecules in experiments using the isolated perfused rat kidney. BSA, bovine serum albumin; cBSA, cationized BSA; ECT, [Asu[1,7]]-eel calcitonin; SOD, recombinant human superoxide dismutase; cSOD; cationized SOD; cBSA, cationized BSA.

tran and polyethylene glycol (PEG) have been used for this purpose.

The most popular macromolecular modifier in this approach is PEG, an inert synthetic polymer, which has been used to modify protein drugs as well as to modify the surface of liposomes. The PEG modification of protein drugs enables them to avoid rapid clearance from the systemic circulation by both a simple size effect in glomerular filtration and a "stealth" effect against recognition by reticuloendothelial systems. Reduced immunogenicity is also an advantage of this approach. Chemical modification using PEG has been studied extensively and the process is called "PEGylation". Clinical trials of protein-PEG conjugates, such as PEGylated adenosine deaminase, superoxide dismutase, asparaginase, and interleukin 2, have been reported (9,10).

Inoue et al. (40) have demonstrated that the blood circulation of a SOD derivative having two molecules of a hydrophobic organic anion, poly (styrene-co-maleic acid) (SM, Mr = 2170), is prolonged by binding to endogenous serum albumin. Although the molecular weight of the derivative is still below the threshold value for glomerular filtration (Mw = 50,000), that as well as removal by reticuloendothelial systems would be restricted by binding to the autologus protein.

Active Targeting Based on Receptor-mediated Mechanisms

In contrast to passive targeting in which only organ level selectivity (first-order targeting) is feasible, the site-specific drug delivery at a cellular or subcellular level (second- or third-order targeting) may be achieved by using active targeting approaches. Macromolecules recognizable by the target cells in a selective manner are used as the carriers for active targeting. Ligand-receptor binding is a typical example of the specific recognition mechanisms in the body. Table II summarizes examples of active targeting with macromolecular carrier systems based on receptor-mediated endocytosis.

Glycoconjugates, including natural glycoproteins and chemically glycosylated macromolecules, have been extensively applied as the ligands for active drug targeting based on receptor-mediated endocytosis. Macromolecules with galactose and mannose residues can be targeted to hepatocytes and macrophages, respectively. In particular, the asialoglycoprotein receptor on hepatocytes seems to have attractive features as a target receptor for drug delivery because of its limited distribution in the body, high binding affinity and rapid ligand internalization. In addition, the free access of galactose-carrying macromolecules to hepatocytes is guaranteed due to the unique microvascular structure of liver pores of up to 100 nm. This feature facilitates active targeting in vivo as well as in vitro (Table II).

Transferrin is also widely applied as a carrier in the active targeting of anticancer agents, proteins and genes to primarily proliferating malignant cells that overexpress transferrin receptors (52). However, most of the successful results obtained with the in vitro and in vivo application of this system are limited. This is probably due to competition with endogenous transferrin, the ubiquitous distribution of transferrin receptor, and anatomical barriers. Macromolecules covalently coupled to folic acid might be used as carriers for rapidly dividing tumor cells in vitro via the endocytic mechanism for this vitamin (53). This carrier system might be advantageous for efficient drug

Table II. Macromolecular Carriers Used for Active Drug Targeting Based on Receptor-mediated Mechanism

Target cell (Receptor)	Carrier	Drug	Results
Hepatocytes (Asialoglycoprotein receptor)	Lactosaminated albumin	Ara-AMP	Therapeutic effect on hepatitis B in clinical trials (reviewed in 41)
	Galactosylated CM-dextran	Ara-C	Selective hepatocyte delivery in vivo (42)
	Galactosylated PLGA	Vitamin K5	In vivo hepatic targeting and pharmacological effect (43)
	Galactosylated HPMA	Doxorubicin	Selective uptake by the liver and hepatoma cells (44)
	Galactosylated SOD	SOD	Therapeutic effect on hepatic ischemia/ reperfusion injury (45)
	Asialoorosomucoid-PLL conjugate	Antisense oligonucleotide	Inhibition of hepatitis B viral gene expression in vitro (46)
		Plasmid DNA	Efficient expression of model and functional genes in vitro and in vivo (reviewed in 46)
	Galactosylated PLL	Plasmid DNA	Long-term factor IX gene expression in vivo (47)
	Galactosylated histone	Plasmid DNA	Efficient model gene expression in vitro (48)
Macrophages (Mannose receptor)	Mannosylated albumin	N-acetylmuramyl dipetide	In vitro and in vivo macrophage activation (reviewed in 49)
	Mannosylated streptavidin	Oligonucleotide (biotinylated)	Enhanced cellular uptake in vitro (49)
	Mannosylated SOD	SOD	Efficient inhibition of superoxide anion release in vitro (50)
			Therapeutic effect on hepatic ischemia/ reperfusion injury (45)
Macrophages (Scavenger receptor)	Maleylated albumin	Daunorubicin	In vitro and in vivo antitumor effect (51)
Tumor cells (Transferrin receptor)	Transferrin	Anticancer agents	Selective cytotoxicity in vitro (reviewed in 52)
Various cells (Transferrin receptor)	Transferrin-PLL-conjugate	Plasmid DNA	Efficient in vitro gene expression (reviewed in 52)
Tumor cells (HL-60) (Folate receptor)	PLL modified with folate	*c-myb* antisense oligonucleotide	Inhibition of cell proliferation in vitro (53)

PLL, poly-L-lysine; PLGA, poly-L-glutamic acid; HPMA, N-(2-hydroxypropyl)methacrylamide copolymer; Ara-AMP, adenine arabinoside monophosphate; Ara-C, cytosine arabinoside; SOD, superoxide dismutase.

delivery to the cytosol compartment because folic acid-macromolecule conjugates are taken up via uncoated pits (caveolae) (54). However, the in vivo potential of this folate-mediated drug targeting remains to be elucidated.

An outstanding feature of receptor-mediated targeting is that significant numbers of approaches have been applied to gene medicines. The advantage of these approaches is only that receptors can efficiently bind and internalize the ligand-DNA complex. Most of the receptors used for this approach however, are not desirable for the cytosol or nuclear delivery of gene medicines. The involvement of ligand degradation in asialoglycoprotein receptor recognition is a typical example. Although gene medicines have been successfully targetied (Table II), the mechanisms of intracellular trafficking of the carrier systems remains to be elucidated to achieve third-order targeting.

Although the intracellular pharmacokinetics of macromolecular carriers are not well understood, there have been several approaches to improving the putative intracellular behaviour of gene medicines. For DNA to escape lysosomal degradation, ligand-polycation conjugates have been combined with adenovirus or fusogenic peptides that can disrupt endosomes. High levels of gene expression in target cells have been achieved with asialoorosomucoid (55) and transferrin carrier systems (52). For efficient translocation to the nucleus, the usefulness of histones, natural cationic DNA-binding proteins containing nuclear localization signals, has been suggested. Although in

vitro gene expression is improved when plasmid DNA is delivered by directly galactosylated histone H1 (48), the mechanism by which the carrier enhanced gene transfer has not been clarified.

Pharmacokinetics of Macromolecular Carriers for Receptor-mediated Targeting

To accomplish in vivo active drug targeting based on receptor-mediated endocytosis, pharmacokinetic considerations are of key importance. However, regardless of several successful approaches using macromolecular carriers of drugs, proteins, oligonucleotides, and genes based on this mechanism (Table II), little pharmacokinetic data regarding this carrier system are available.

The efficacy of active targeting carrier systems at the organ level can be also discussed based on the clearance concept as described in the section on passive targeting. Pharmacokinetic studies on receptor-mediated targeting to the hepatocytes via galactose recognition mechanism have been performed (56,57). Figure 5 shows the hepatic and urinary clearance of synthesized glycoconjugates with a galactose-terminal acting as drugs or carriers. Proteins with various molecular weights, carboxymethyl dextran and poly-L-glutamic acid were galactosylated using 2-imino 2-methoxyethyl 1-thioglycoside (58). The results showed that the galactose modification increased the hepatic uptake of each macromolecule to various degrees depending on the backbone type. The clearance of relatively larger proteins such as IgG, BSA, and SOD, are almost identical to the theoretical maximum value (85,000 μl/hr; hepatic plasma flow rate in mice). Selective delivery to the liver parenchymal cells was also confirmed by cell separation experiments using collagenase. Based on the results, ara-C, vitamin K5, and SOD have been targeted to hepatocytes (Table II). One advantage of the glycosylation applied in these studies is that the net charge of macromolecules is not altered by the modification. In contrast, proteins synthetically glycosylated with p-aminophenyl derivatives of sugars are recognized by non-parenchymal liver cells via the scavenger receptor due to an increase in the net negative charge (59).

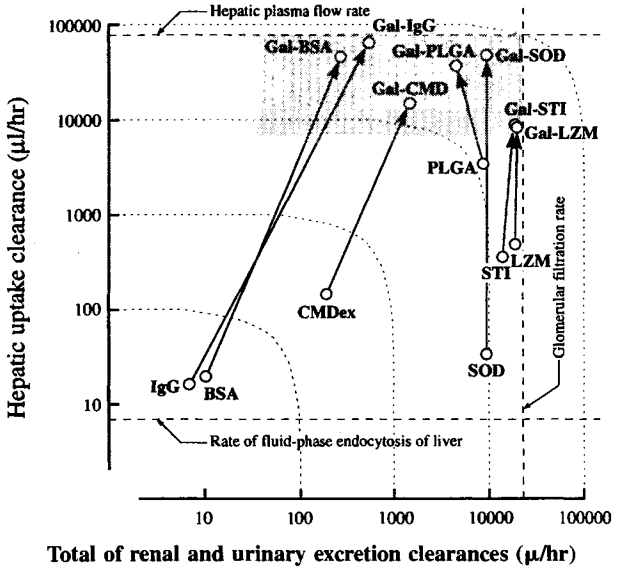


Fig. 5. Hepatic uptake and urinary excretion clearances of galactosylated macromolecules in mice after an intravenous injection at a dose of 1 mg/kg. IgG, immunoglobulin G; BSA, bovine serum albumin; SOD, recombinant human superoxide dismutase; STI, soybean trypsin inhibitor; LZM, lysozyme; CMDex, carboxymethyl-dextran; PLGA, poly-L-glutamic acid. Arrows indicate the effect of galactosylation of the original macromolecules.

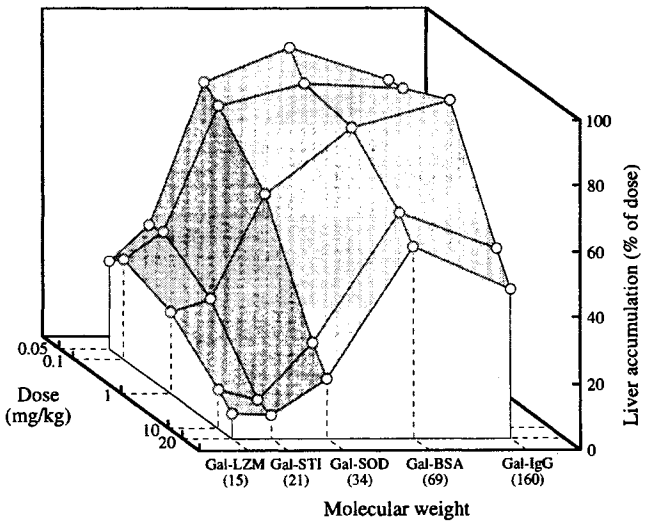


Fig. 6. Effects of dose and molecular weight on the hepatic targeting efficacy of galactosylated proteins. Liver accumulation at infinite time was calculated by the equation (CLliver/CLtotal) × 100 (%). Circles represent the accumulation of galactosylated proteins at an doses from 0.05 to 20 mg/mg.

The administration dose is a very important factor in these phenomena since active targeting employing receptor-mediated endocytosis is saturable and non-linear, while passive targeting is essentially a linear processes over a wide range of doses. Figure 6 summarizes the hepatic targeting efficacy of five galactosylated proteins at five different doses. Larger glycoprotein derivatives accumulated more in the liver (about 70–80% of dose) at doses below 1 mg/kg. On the contrary, smaller glycosylated proteins showed relatively small values even at low doses. The final hepatic accumulation was limited to about 30 % of the dose due to their large urinary clearance.

Taken together, these results suggest that molecular weight of the carriers should be controlled to satisfy the following conditions. A satisfactory number of galactose molecules per protein molecule should be introduced to ensure a high affinity for the receptor (57). The major clearance process of glomerular filtration should be minimized since targeting efficacy to the hepatocytes is decided by the balance of hepatic uptake and urinary excretion clearance. However, the results showed that, even if a protein such as SOD (Mw = 32,000) susceptible to glomerular filtration is tested, effective hepatocyte targeting in vivo is still possible when endowed with high affinity for the receptor and administered at an appropriate dose. More than 80% of injected galactosylated SOD was delivered at lower doses and this can be simply explained by differences in the maximum clearance values in hepatic uptake and glomerular filtration in mice, namely, 85,000 vs. 20,000 μl/hr, respectively. Thus, pharmacokinetic studies are useful to clarify the meaning

of the in vivo disposition of ligands based on receptor-mediated endocytosis.

In addition, there is a more crucial size limitation in galactose-carrying carriers. There is an upper limit for recognition by the asialoglycoprotein receptor, that is, a ligand larger than 15 nm is specifically cleared by the galactose receptor on the surface of Kupffer cells (12). Accordingly, Perales et al. (47) have suggested that size (10–12 nm) of DNA/ligand-PLL complexes is key to successful in vivo gene expression for up to 140 days with this system.

Active Targeting with Monoclonal Antibodies

Antigen-antibody interaction is another specific recognition system which can be applied for active targeting. The advent of monoclonal antibody technology has reawakened the "magic bullet" concept proposed by Ehrlich at the beginning of this century especially in cancer chemotherapy, whereby anticancer agents can be specifically targeted to tumors (3). Although a great deal of effort has been directed towards using monoclonal antibodies as carriers for anticancer drugs and prodrug-activating enzymes including clinical trials, the primary goal has not yet been realized (for a review, see Ref. 3). As far as in vivo targeting efficacy to solid tumors is concerned, the advantage of this approach over passive targeting has not been explored (Table I), while a protocol for the rational design of carrier systems has been proposed (30). Although a variety of factors are involved, passive processes such as extravasation across the tumor capillaries and transport in the tumor tissue will limit the accessibility of monoclonal antibodies to the target cells before they can recognize an epitope on the tumor cell surface.

An alternative approach to active tumor targeting with monoclonal antibody is to directly attack the endothelial cells of the tumor vascular bed. This would circumvent drawbacks in relation to the inaccessibility of the carrier. Immunotoxin directed to tumor vascular endothelium causes complete occlusion of the tumor vasculature and dramatic tumor regression in a mouse model (60). Furthermore, the vasoactive cytokine interleukin 2 conjugated with a monoclonal antibody against a basement membrane antigen of tumor vessels can enhance the delivery of successively administered macromolecules to the tumor tissue (61).

Active targeting to the brain has also been achieved with a monoclonal antibody directed to the transferrin receptor (62), since the brain microvessel endothelial cells have high concentrations of this receptor. A monoclonal antibody designated as OX26, which binds to an extracellular projecting epitope on the receptor and which undergoes receptor-mediated transcytosis, has been used as a carrier for drugs, peptides, oligonucleotides, and peptide nucleic acids (62). The usefulness of this carrier has been shown in terms of absolute activity in penetrating the barrier as well as pharmacokinetics at the whole body level. A monoclonal antibody against the human insulin receptor has a similar level of potency.

Monoclonal antibodies have also been used as DNA carriers. Efficient in vitro gene transfer has been achieved for carrier systems of PLL conjugated with antibodies or fragments directed against the polymeric immunoglobulin receptor (63), the epidermal growth factor receptor (64), and the CD3-T cell receptor complex (65). The in vivo potential remains to be elucidated.

CONCLUSIONS AND PERSPECTIVE

Despite considerable progress in the basic studies of targeted delivery with macromolecular carriers, practical application remains extremely limited. PEGylated protein drugs, such as PEG-adenosine deaminase for severe combined immunodeficiency and PEG-asparaginase for acute lymphocytic leukemia, were approved by FDA in 1990 and 1994, respectively. As to anticancer agents, recent results in clinical trials do not warrant optimism in applying this modality for tumor targeting (3). Neocarzinostatin conjugated with poly(styrene-co-maleic acid), designated as SMANCS, is a special example. SMANCS combined with Lipiodol®, a lipid contrast medium that shows prolonged retention in tumor tissue, has been officially approved in Japan as a chemotherapeutic agent for hepatoma in 1993. Promising results in clinical trials have also been obtained for doxorubucin-N-(2-hydroxypropyl)methacrylamide (HPMA) copolymer conjugate (30) and neocarzinostatin-monoclonal antibody conjugate (66). For gene medicines, the use of macromolecular carrier systems has been limited to animal studies.

This review considered recent advances in the targeted delivery of various types of drugs using macromolecular carriers particularly from a pharmacokinetic point of view. The in vivo application of macromolecular carriers for site specific drug delivery requires not only affinity for a specific target but also favorable general pharmacokinetic properties in the body at the organ, cellular, and subcellular levels. In addition, one of the most critical problems inherent in the use of macromolecular carrier systems especially protein carriers like antibodies, is the occurrence of adverse immunological reactions. For example, human anti-mouse antibody has been produced by the host in most clinical trials using murine monoclonal antibodies. Although this problem can be overcome by useing less immunogenic protein carriers prepared by genetic engineering such as, chimeric, humanized or totally human proteins, this issue should be crucial for all types of drug/carrier complexes.

The keys to the clinical success of targeted delivery with macromolecular carrier systems involve the chemical and physicochemical rationalization of the design of drug-macromolecuie complexes, understanding of the physiology of the human body, the assessment of pharmacokinetic properties of macromolecular carrier systems in humans, the elucidation of the intracellular pharmacokinetic mechanisms of macromolecules, and the estimation and prevention of toxicity and antigenicity of the macromolecular carrier systems. Therefore, the development of macromolecular carrier research depends greatly on the progress in a variety of research fields involving biochemistry, immunology, cell and molecular biology, pharmacokinetics, pharmacology. The integration of simultaneous progress in multidisciplinary research should accelerate the emergence of novel macromolecular carrier systems in the market place.

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