Pharmaceutical Research, Vol. 7, No. 4, 1990

Research Article

Disposition Characteristics of Macromolecules in Tumor-Bearing Mice

Yoshinobu Takakura,[1,2] Takuya Fujita,[1] Mitsuru Hashida,[1] and Hitoshi Sezaki[1]

Received April 25, 1989; accepted November 6, 1989

As part of the strategy for the design of macromolecular carriers for drug targeting, the disposition characteristics of macromolecules were studied in mice bearing tumors that served as target tissues. Eight kinds of macromolecules including four polysaccharides and four proteins with different molecular weights and electric charges were used; tissue distribution and tumor localization after intravenous injection were studied. Pharmacokinetic analysis revealed that the tissue radioactivity uptake rate index calculated in terms of clearance was different among the tested compounds; especially, the urinary radioactivity excretion clearances and the total hepatic radioactivity uptake clearances varied widely. Compounds with low molecular weights (approximately 10 kD) or positive charges showed lower tumor radioactivity accumulation; radioactivity was rapidly eliminated from the plasma via rapid urinary excretion or extensive hepatic uptake, respectively. On the other hand, large and negatively charged compounds, carboxymethyl dextran, bovine serum albumin, and mouse immunoglobulin G, showed higher radioactivity accumulation in the tumor (calculated total amounts were 15.6, 10.8, and 20.8% of the dose, respectively) and prolonged retention in the circulation. These results demonstrated that the total systemic exposure rather than the uptake rate index was correlated with total tumor uptake. Molecular weight and electric charge of the macromolecules significantly affected their disposition characteristics and, consequently, determined radioactivity accumulation in the tumor. It was concluded that a drug–carrier complex designed for systemic tumor targeting should be polyanionic in nature and larger than 70,000 in molecular weight.

KEY WORDS: Macromolecular compounds; physicochemical characteristics; tumor targeting; systemic disposition; hepatic uptake; urinary excretion.

INTRODUCTION

Further progress in chemotherapy will depend on optimization of drug delivery. Interest has recently focused on the targeting of highly potent antitumor drugs and biologically active peptides which may have undesirable side effects detrimental to nontarget cells. Various drug carrier systems have been developed for site-specific delivery of these drugs (1). Among them, conjugation of drugs to macromolecules seems to be one of the most promising since macromolecules are highly diverse in their physicochemical properties and functions (2–4).

The rationale for this approach is that conjugation of a drug with a macromolecule may alter its disposition properties depending on the properties of the carrier macromolecule. However, little systematic information about the disposition properties of macromolecules, including drug conjugates, has been reported.

In our series of investigations, several kinds of dextran conjugates of an antitumor antibiotic, mitomycin C (MMC) (5–17), and model peptide drugs such as soybean trypsin inhibior (STI, 20 kD) (18,19) and uricase (UC; 120 kD) (20) were synthesized, and their physicochemical, pharmacodynamic, and pharmacokinetic characteristics were systematically examined. These studies revealed that the disposition properties of drugs can be controlled by the selection of the physicochemical characteristics of the carrier, such as molecular weight and electric charge.

In the present study, dextrans and proteins (Table I) were selected as model macromolecules and also as candidates for drug carriers, and their disposition properties were studied in mice bearing tumors that served as target tissues. The purpose of this study was to clarify the general relationship between the physicochemical characteristics and *in vivo* behavior of selected macromolecules and to construct the strategy for the design of drug–macromolecular conjugates.

MATERIALS AND METHODS

Chemicals

Dextrans with average molecular weights of about 10 kD (T-10) and 70 kD (T-70) were purchased from Pharmacia, Uppsala, Sweden. Bovine serum albumin (BSA; Fraction V) was obtained from Armour Pharmaceutical Co., U.K. Apoprotein of neocarzinostain (apoNCS) and mouse immunoglobulin G (IgG) (monoclonal antibody A7 against human

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

0724-8741/90/0400-0339$06.00/0

Table I. Physicochemical Characteristics of Model Macromolecules

Compound[a]	MW (original)	Electric charge	Type of labeling
Dextran(T-10)	9,900	Neutral	^{14}C
Dextran(T-70)	64,400	Neutral	^{14}C
DEAED(T-70)	64,400	Positive	^{14}C
CMD(T-70)	64,400	Negative	^{14}C
apoNCS	10,700	Negative	^{111}In
BSA	66,000	Negative	^{111}In
cBSA	66,000	Positive	^{111}In
IgG	150,000	Negative	^{111}In

[a] DEAED, diethylaminoethyldextran; CMD, carboxymethyldextran; apoNCS, apoprotein of neocarzinostatin; BSA, bovine serum albumin; cBSA, cationized BSA; IgG, mouse immunoglobulin G.

colon carcinoma cells) were gifts from POLA Cosmetics, Tokyo, and from Dr. Takahashi, Kyoto Prefectural University of Medicine, Kyoto, Japan, respectively. Potassium [^{14}C]cyanide (798.18 μCi) and indium chloride ([^{111}In]Cl_3) were supplied by Amersham Japan, Tokyo, and Nihon Mediphysics, Takarazuka, Japan, respectively. All other chemicals were obtained commercially as reagent-grade products.

Preparation of Charged Model Macromolecules

Diethylaminoethyl-dextran [DEAED (T-70), Fig. 1] was synthesized by a published method (21). Dextran(T-70) (1 g) was dissolved in 2.17 *M* NaOH solution (15 ml) and (diethylamino)ethyl chloride hydrochloride (2 g) was slowly added. The mixture was kept at 80°C for 4 hr with constant stirring. To synthesize carboxymethl-dextran [CMD(T-70), Fig. 1] (22), dextran(T-70) (1 g) was dissolved in 6 *M* NaOH solution (8.3 ml) and monochloracetic acid (2 g) was slowly added. The mixture was maintained at 70°C for 20 min with stirring. The product was dialyzed extensively against distilled water and then concentrated by ultrafiltration. Radiolabeled DEAED and CMD were synthesized as described above using ^{14}C-labeled dextran(T-70). The final products were chromatographed on a Toyoperal HW-60S (TOYO, Tokyo) column (2.4 × 65 cm) and confirmed to be almost identical to the original dextran(T-70) in molecular size. The molecular charges were checked by a batch method using a CM-Sephadex cation exchanger and a DEAE-Sephadex A-50 anion exchanger (Pharmacia, Uppsala, Sweden) as described previously (13). Cationized BSA (cBSA, Fig. 1) was synthesized according to the method of Pardridge *et al.* (23,24). Five milliliters of a 10% solution of BSA in distilled water was slowly added to 30 ml of 2 *M* hexamethylenediamine at pH 6.5 After 30 min and 1 hr, 0.5 g of 1-ethyl(3-dimethylaminopropyl) carbodiimide hydrochloride was added and the pH of the solution was kept at 6.5 by addition of a 1 *N* HCl solution. The reaction was allowed to proceed overnight with stirring and then dialyzed extensively against distilled water. The protein ws purified by chromatofocusing using the polybuffer exchanger 94 resin and the polybuffer 96 elution buffer (Pharmacia, Uppsala, Sweden). The major protein peak eluted in the void volume, indicating that the *p*I was more than 9.0, and was collected and concentrated by ultrafiltration. A Toyopearl HW-60S column was employed for molecular size estimation, and cBSA and BSA had nearly identical molecular sizes. The cationic nature of cBSA was confirmed by its adsorption on a CM-Sephadex C-50 anion exchanger.

Radiolabeling of Dextrans

[*Carboxyl*-^{14}C]dextran(T-10) and [*carboxyl*-^{14}C]dextran (T-70) were prepared according to the method of Isbell *et al.* (25) with slight modifications. Briefly, 0.05 mmol of dextran(T-10), sodium bicarbonate, and sodium hydroxide was dissolved in 5 ml of distilled water and frozen in a glass tube. Then potassium [^{14}C]cyanide (0.05 mmol) was added and the tube was sealed in a flame. In the case of dextran(T-70), 0.02 mmol of dextran and other reagents was dissolved in 10 ml of distilled water. The mixture was thawed and stored at 45°C for 24 hr, then heated for 7 hr at 50°C in a stream of air to effect hydrolysis. The product was purified by gel filtration using a Sephadex G-25 (Pharmacia, Uppsala, Sweden) column and concentrated by ultrafiltration. The specific activities of dextran(T-10) and dextran(T-70) were approximately 2.2 and 0.3 μCi/mg, respectively.

Radiolabeling of Proteins.

Proteins were labeled with ^{111}In using the bifunctional

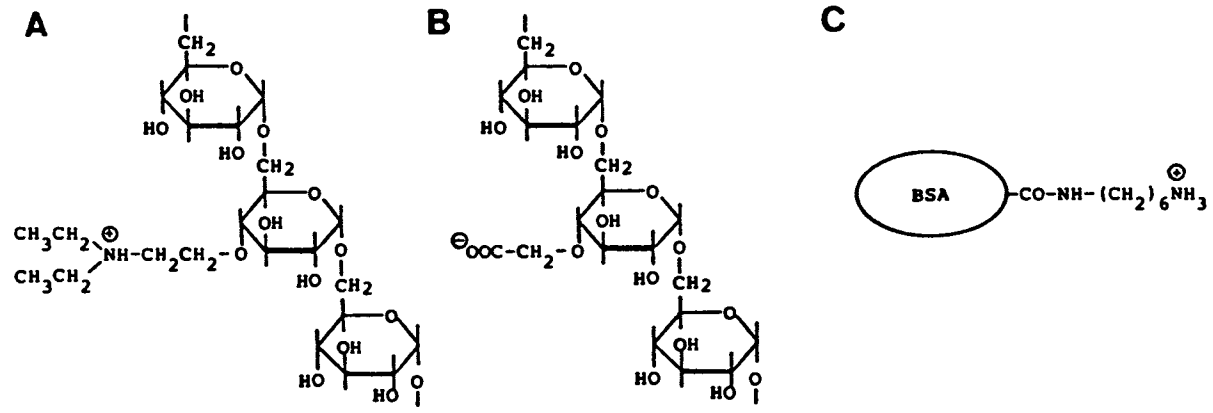

Fig. 1. Chemical structure of model macromolecules. (A) Diethylaminoethyldextran (DEAED); (B) carboxymethyldextran (CMD); (C) cationized bovine serum albumin (cBSA).

chelating agent diethylentriaminopentaacetic acid (DPTA) anhydride according to the method of Hnatowich *et al.* (26). The protein (10 mg) was dissolved in 1 ml of 0.1 *M* *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7.0) and equimolar DPTA anhydride (Dojindo Laboratories, Kumamoto, Japan) in 10 μl of DMSO was added. The mixture was stirred for 30 min at room temperature and purified by gel filtration with Sephadex G-25 to remove the free DTPA. The protein was concentrated by ultrafiltration. Forty microliters of $^{111}InCl_3$ solution (80 μCi) was added to 40 μl of 1 *M* sodium acetate buffer (pH 6.0), and then 80 μl of DTPA-coupled protein solution was added to the mixture. At 30 min after addition, the mixture was purified by gel filtration (Sephadex G-25) using normal saline as an eluent, and the protein fractions were collected and concentrated by ultrafiltration. The specific activities of proteins were approximately 0.2 mCi/mg.

Animals and Tumors

Male ddY mice (22–25 g) were obtained from the Shizuoka Agricultural Co-operative Association for Laboratory Animals, Shizuoka, Japan. Sarcoma 180 (S180) was maintained in ddY mice by weekly intraperitoneal transfer of 10^8 cells obtained from ascitic fluid.

Procedure for the Animal Experiment

S180 cells (10^7) suspended in 0.1 ml of Hanks' balanced salt solution were inoculated in the dorsal subcutaneous tissue of ddY mice. Twelve days after inoculation, tumor-bearing mice (28–32 g; tumor weight, 0.5–1.2 g) received a 100-mg/kg (3-μCi/kg) dose of radiolabeled test macromolecules (appropriately diluted with unlabeled test material) in saline by tail vein injection and were then housed in metabolic cages for urine collection. Blood was collected from the vena cava completely and the mice were killed at 0.17, 1, 8, and 24 hr postdose. In addition, the heart, lung, liver, spleen, kidney, intestine, muscle, iliac lymph nodes (three or four nodes), and tumor were excised, rinsed with saline, weighed, and subjected to assay. Four mice were sacrificed at each sampling time.

Analytical Methods

The procedure for quantitation of ^{14}C radioactivity was a modification of the method of Mahin and Loftberg (27). Minced and weighed tissues and untreated plasma and urine (less than 0.2 g) were put into a counting vial, and 0.2 ml of perchloric acid (60%) and hydrogen peroxide (35%) was added. The resulting mixture was heated at 70°C for 90 min with agitation. After cooling to room temperature, 10 ml of scintillation medium (Clear-sol I, Nacalai Tesque, Kyoto, Japan) was added and the radioactivity was determined in a liquid scintillation system (LSC-900, Aloka Co, Tokyo). The ^{111}In radioactivities were counted untreated in a well-type NaI-scintillation counter (ARC-500, Aloka Co, Tokyo). The plasma volume of each organ was determined from the ^{14}C-CMD data at 1 hr after injection and used to correct for the contamination of each organ with plasma.

Data Analysis

The tissue distribution data were evaluated using a tissue uptake rate index calculated in terms of clearance as reported previously (14). The change in the amount of radioactivity in a tissue with time can be described as follows:

$$dT(t)/dt = Cl_{in} \times C(t) - K_{out} \times T(t) \quad (1)$$

where $T(t)$ (% of dose/g) is the amount of radioactivity in 1 g of the tissue, $C(t)$ (% of dose/ml) is the plasma concentration of radioactivity, Cl_{in} (ml/hr/g) is the tissue uptake rate index (clearance) from the plasma to the tissue, and K_{out} (1/hr) is the efflux rate constant from the tissue. In the present study, the efflux processes of the radioactivity, such as its return to plasma from the tissue, may be negligible during the time studied, since the radioactivity derived from the macromolecule may be retained in the tissue for a considerably long period (28–30). Ignoring efflux, Eq. (1) integrates to

$$Cl_{in} = T(t_1) \Big/ \int_0^{t_1} C(t)dt = T(t_1)/AUC_{0-t_i} \quad (2)$$

According to Eq. (2), the tissue uptake rate index is calculated using the amount of radioactivity in the tissue at any time and the area under the plasma concentration–time curve (AUC) up to that time. Then the organ clearance (CL_{org}) is expressed as follows:

$$CL_{org} = Cl_{in} \times W \quad (3)$$

where W (g) is the total weight of the organ. Urinary clearance (CL_{urine}) was calculated by Eq. (2) using the accumulated amount excreted in urine. In addition, the total-body clearance (CL_{total}) equals the sum of each organ clearance and the following equation should hold:

$$\begin{aligned} CL_{total} &= dose/AUC \\ &= CL_{urine} + CL_{liver} + CL_{kidney} \\ &\quad + \text{---} + CL_{tumor} \end{aligned} \quad (4)$$

Furthermore, the total tumor accumulation at infinite time was calculated to compare the amount of tumor radioactivity uptake among compounds according to the following equation, assuming the tumor weight to be 1 g:

$$\text{total tumor accumulation (\% of dose)} = (Cl_{tumor} \times 1)/CL_{total} \times 100 \quad (5)$$

RESULTS

Tissue Distribution and Urinary Excretion of Dextrans

After iv injection of ^{14}C-dextran(T10) to S180-bearing mice, an extremely rapid elimination of radioactivity in the plasma was observed; approximately 84% of the radioactivity dose was excreted in the urine within 8 hr (data not shown). Radioactivity concentrations in all tissues were very low. ^{14}C-Dextrans of 70,000 molecular weight showed relatively slower elimination of radioactivity from the plasma. The tissue distribution and urinary excretion of radioactivity were dependent on the dextran's electric charge. Figure 2 shows the tissue distributions of ^{14}C-DEAED(T-70) and ^{14}C-CMD(T-70) after iv injection. Radioactivity derived from ^{14}C-DEAED(T-70) was rapidly cleared from the plasma, and

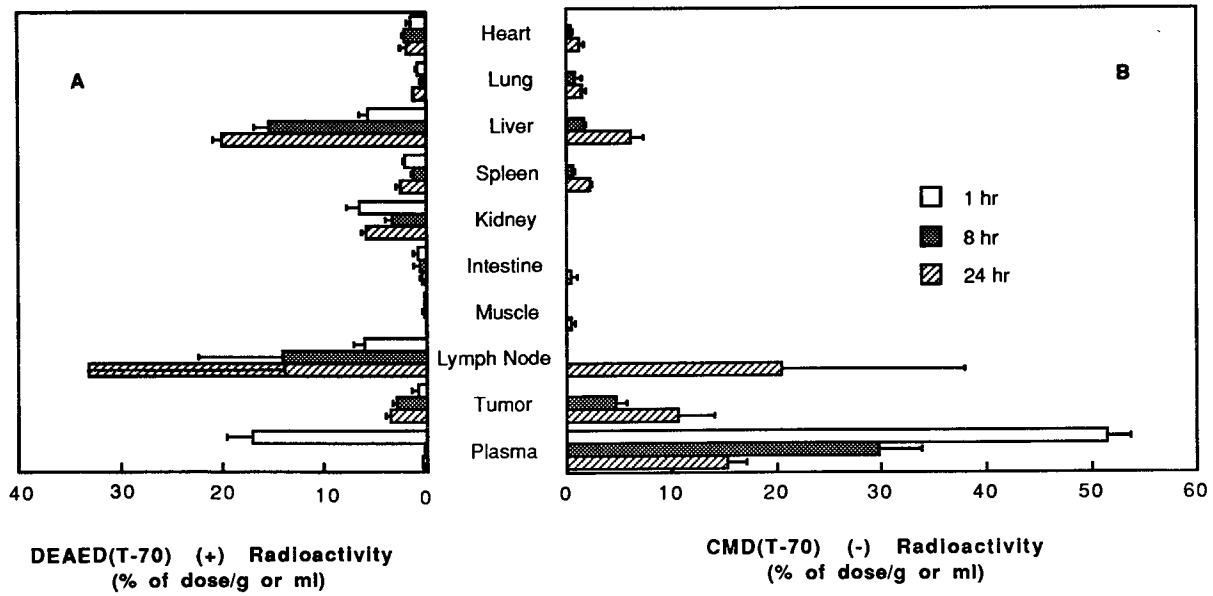

Fig. 2. Tissue distribution of radioactivity for ^{14}C-DEAED(T-70) (A, positive charge) and ^{14}C-CMD(T-70) (B, negative charge) after iv injection in S180-bearing mice. Results are expressed as the mean ± SD of four mice.

a significant accumulation of radioactivity was observed in the liver and lymph node. A small amount of radioactivity was observed in the tumor. In contrast, after anionic ^{14}C-CMD(T-70) administration, radioactivity was retained in the plasma much longer, and liver radioactivity was lower. Somewhat increased accumulation of radioactivity was observed in the tumor at 24 hr. Neutral ^{14}C-dextran(T-70) was intermediate in its distribution profile (data not shown). Urinary recovery of radioactivity over 24 hr after injection was 33.5, 54.2, and 45.4% for ^{14}C-dextran(T-70), ^{14}C-DEAED(T-70), and ^{14}C-CMD(T-70), respectively.

Tissue Distribution and Urinary Excretion of Proteins

The plasma elimination of radioactivity after iv administration of ^{111}In-apoNCS was very rapid; approximately 74% of the radioactivity dose was recovered in urine within 2 hr after iv injection (data not shown). No significant accumulation was observed in any tissue. Figure 3 shows the tissue distributions of ^{111}In-cBSA and ^{111}In-BSA after iv injection. Radioactivity was principally retained in the plasma for a long time after anionic ^{111}In-BSA administration. Most of the organs sampled accumulated significant amount of

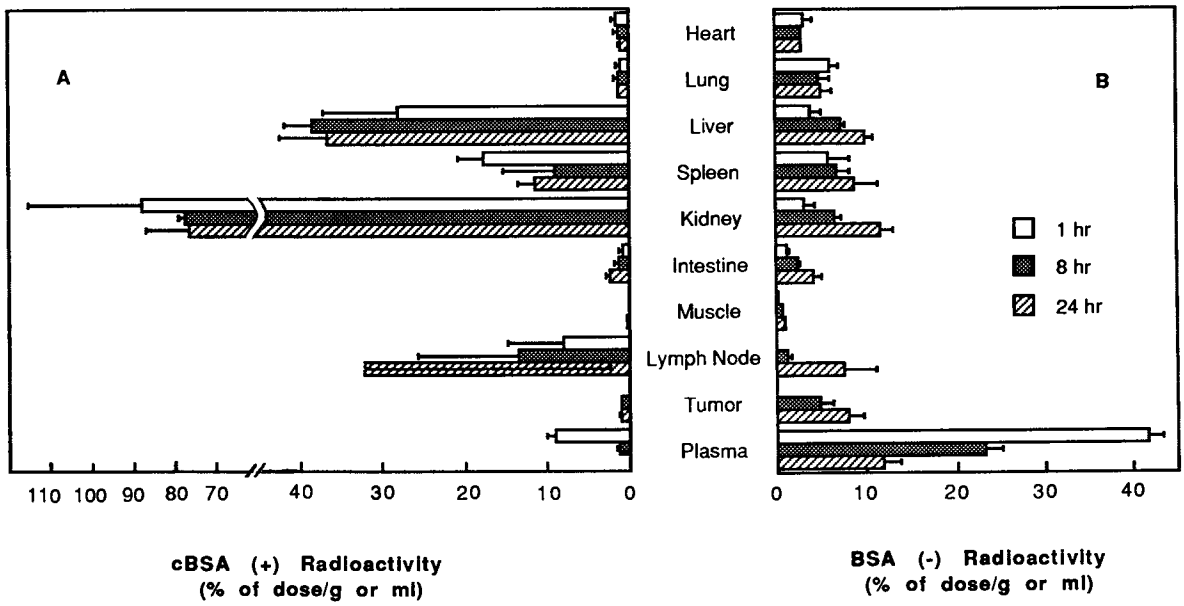

Fig. 3. Tissue distribution of radioactivity for ^{111}In-cBSA (A, positive charge) and ^{111}In-BSA (B, negative charge) after iv injection in S180-bearing mice. Results are expressed as the mean ± SD of four mice.

radioactivity. In contrast, cationic ^{111}In-cBSA was rapidly cleared from the plasma and remarkable accumulations of radioactivity were observed in the liver, kidney, and lymph node; tumor levels remained low. The urinary excretion of radioactivity after ^{111}In-BSA and ^{111}In-cBSA administration were 4.7 and 6.1% within 24 hr after injection, respectively, ^{111}In-IgG exhibited a distribution pattern similar to that observed for ^{111}In-BSA; urinary recovery of radioactivity over 24 hr was only 1.6% of the dose (data not shown).

Pharmacokinetic Analysis

Table II summarizes the AUCs, total-body and urinary clearances, and tissue uptake rate indices for representative organs after administration of each model macromolecule. Pharmacokinetic analysis revealed that there were differences in the tissue uptake rate index among model compounds; compounds with low molecular weights showed the highest values; positively charged macromolecules also showed relatively high values. These differences were particularly pronounced for the liver and kidney. The urinary excretion clearances also varied to a great extent. Compounds with low molecular weight such as dextran(T-10) and apoNCS, had extremely large urinary clearances. Cationic DEAED and cBSA showed large tissue uptake rate indices in the liver, spleen, and kidney. To compare disposition characterisitcs among the radiolabeled compounds, the absolute value of total-body clearance and the contribution of organ clearances to it are shown in Fig. 4. It is obvious that the large total-body radioactivity clearances associated with the small compounds depended mainly on their large urinary clearances. For the larger molecules, negatively charged macromolecules appeared to have small total-body radioactivity clearances, while positively charged molecules were associated with large values. Neutral dextran was intermediate.

Total Tumor Accumulation

Figure 5 shows the calculated total tumor accumulation of radioactivity for the test compounds at infinite time after injection into S180-bearing mice. Small values were obtained for small, positively charged or neutral macromolecules. Compounds with negative charges showed a greater total tumor accumulation of radioactivity, i.e., 15.6% for CMD(T-70), 10.8% for BSA, and 20.8% for IgG.

DISCUSSION

To achieve effective drug targeting, it is necessary to control not only uptake by the target tissue but also the general disposition characteristics of the targeted compound. In the present study, the disposition characteristics of radiolabeled model macromolecular compounds were studied in S180-bearing mice to construct a strategy for targeting with drug–macromolecule conjugates.

It is important to select an appropriate method of radiolabeling of macromolecular compounds for accurate estimation of their *in vivo* disposition. In the present study, dextrans and proteins were labeled with ^{14}C and ^{111}In, respectively (Table I). The net tissue uptake could be estimated since the radioactivity in the macromolecule would be retained for a long period in the tissue after distribution. Dextran is known to be unsusceptible to metabolic degradation and histochemical studies demonstrated that dextran accumulated in some tissues of mice for several days after iv injection (28). ^{14}C-Labeled dextran was also shown to be resistant to degradation in the liver of mice (29). Radioiodination is the most common method for protein labeling, but radioactivity may return to the blood circulation if the labeled protein was degraded in the organ. ^{111}In labeling was deemed preferable for the disposition study because ^{111}In is reported to be accumulated in the organ by exchange into an iron-binding protein (30) after intracellular degradation.

The liver appeared to be the most important organ for the systemic disposition of cationic and neutral macromolecules. Liver uptake rate indices of anionic compounds were small (Table II), and these values were similar to those reported for polyvinylpyrrolidon, which is reportedly taken up by the liver by fluid-phase endocytosis (31). IgG and BSA are serum-derived proteins and factors other than physicochemical properties might affect their liver uptake. However, an anionic nature seems to play an important role in slow uptake of macromolecules since CMD(T-70) had a small liver uptake rate index similar to those observed for serum proteins. Cationic macromolecules were taken up rapidly by the liver (Table II) and the importance of this process in their total disposition was shown (Fig. 4). In contrast to

Table II. AUCs, Clearances, and Tissue Uptake Rates for Radioactive Model Macromolecules in S180-Bearing Mice

Compound	AUC (% of dose · hr/ml)	Clearance (μl/hr)		Tissue uptake rate index (μl/hr/g)				
		CL_{total}	CL_{urine}	Liver	Spleen	Kidney	Muscle	Tumor
^{14}C-Dextran(T-10), neutral	6.6	15,200	12,800	191	26.6	210	65.4	236
^{14}C-Dextran(T-70), neutral	146	685	257	190	20.5	7.7	2.5	23.9
^{14}C-DEAED(T-70), +	51.1	1,960	1,060	291	33.3	59.3	6.7	59.1
^{14}C-CMD(T-70), −	1010	99.1	66.1	9.0	3.0	0.6	1.1	15.5
^{111}In-apoNCS (10) −	3.8	26,200	20,000	27.0	19.7	1,050	26.6	132
^{111}In-BSA (60) −	764	131	8.5	18.5	15.6	21.2	1.8	14.2
^{111}In-cBSA (60) +	48.2	2,080	166	1,000	233	1,970	5.4	26.9
^{111}In-IgG (150) −	1620	61.6	2.4	17.4	5.4	4.6	1.2	12.8

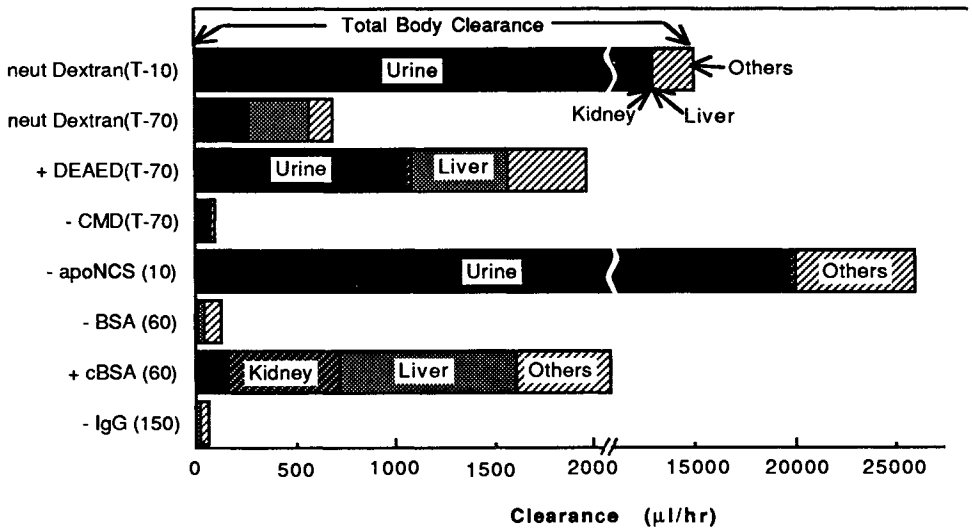

Fig. 4. Contribution of organ clearances to total-body clearance of radioactivity after iv administration of radiolabeled macromolecules in S180-bearing mice.

most other organs in which the capillary presents a substantial barrier between the vascular and the interstitial spaces, the liver has dicontinuous endothelial capillaries and this structure brings circulating substances in plasma into free contact with the surface of hepatocytes. This anatomy character explains the electrostatic interaction of cationic macromolecules with the negative charges of the liver cell surfaces, as reported for cationic MMCDs (16,17). The uptake by the spleen could also be explained by the same mechanism.

Remarkable radioactivity accumulation was observed in the lymph nodes after iv administration of all macromolecules tested except BSA. The mechanism remains obscure, but macromolecules might be useful as drug carriers to prevent lymph node metastasis of tumors by systemic or local injection (9).

Urinary excretion appeared to be the predominant elimination route for radioactivity associated with some of the macromolecules. It is well known that the glomerular capillary wall functions as a barrier based on discrimination of molecular size and electric charge (32). Neutral dextrans wth molecular radii less than 20 Å are reported to cross the glomerulus without measurable restriction (33). It is also reported that negatively charged dextran sulfate is restricted to a great extent, while positively charged DEAED showed enhanced ability to cross the glomerular wall compared with neutral dextrans of similar size (33).

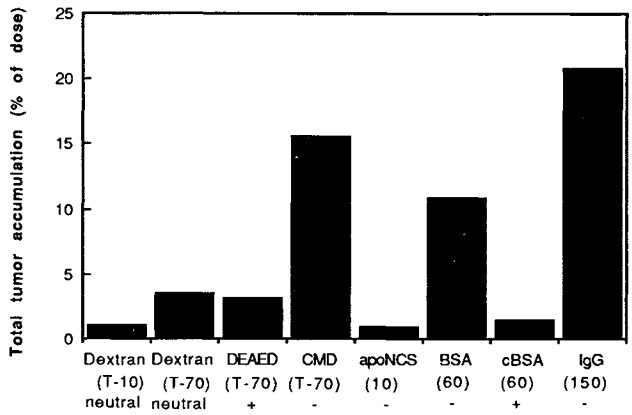

Fig. 5. Total tumor accumulation of radioactivity for macromolecules at infinite time after iv injection in S180-bearing mice.

The effect of molecular size on urinary excretion was obvious; compounds with low molecular weights showed extremely large urinary excretion clearances, comparable to the glomerular filtration rate in mice (34). Urinary excretion of these compounds was shown to be the main factor in their disposition (Fig. 4). Furthermore, the difference in the renal disposition characteristics between the globular protein BSAs and linear polysaccharide dextrans was also shown. BSAs had smaller urinary clearances but larger kidney uptake rate indices than comarable dextrans. Dextrans seemed to more easily pass through the glomerular capillary wall. The interaction of cBSA with the glomerular wall (35) and its reabsorption in the renal proximal tubule (36) have been reported; these might explain the observed radioactivity accumulation in the kidney.

In the present study, a tumor was considered as a target tissue since antitumor agents have often been chosen as the object of macromolecular conjugation (2–4). Generally, tumor tissues are characterized by enhanced vascular permeability and the lack of a lymphatic system (37). These characteristics may explain why some macromolecules are reported to be accumulated in the tumor tissue after iv injection (38,39). Administration of all of the maromolecules led to accumulation of radioactivity in tumor tissue. Compounds with a low molecular weight or a positive charge showed relatively larger tumor uptake rates. Great differences were also observed in the total uptake (Fig. 5). These results demonstrated that the tumor uptake rate did not relate to the tumor uptake amount.

IgG associated radioactivity accumulated in the tumor to the greatest extent among the compounds tested. Thus, IgG was suggested to be a suitable carrier for passive tumor targeting; it is a negatively charged macromolecule, has a large molecular weight, and persists for long periods of time

in the circulation. In the case of target-specific IgG, effective active targeting would be expected.

It has been reported that a polymeric prodrug of MMC with a negative charge, MMCDan(T-70), exhibited superior tumor uptake and antitumor activity against S180 (14). In contrast, cationic MMCDcat failed to accumulate in the tumor, probably due to its rapid hepatic uptake and urinary excretion. Figure 6 summarizes the hepatic and urinary clearances of the eight model macromolecular compounds and the MMCDs. It appeared that a general relationship exists between the physicochemical and the disposition characteristics of macromolecules. Small molecules were characterized by large urinary clearances possibly regardless of molecular charge. Although small positively charged molecules were not tested in this study, they would have similar high urinary clearances as conjectured from the results for MMCDcat(T-10) in rats (8,14). For larger molecules with molecular weights greater than approximately 70,000 molecular charge seemed to be important. Anionic compounds had small hepatic and urinary clearances. on the other hand, positively charged molecules showed much higher values in both clearances. Extrapolating from the data for MMCDcat(T-500) (8,14) and cationized IgG (40), similar high hepatic clearances but lower urinary clearances would be expected for larger compounds with positive charge. Neutral dextran showed the intermediate clearance values; this might be the case for similar or larger macromolecules.

cBSA was reported to be a useful carrier for the targeting of a peptide drug to the brain *in vitro* (23,24) and *in vivo* after intracarotid injection (23). However, cBSA showed a very low comprehensive availability to the tumor (Fig. 5). In addition, minimal radioactivity was detected in the brain after iv injection in this study (data not shown).

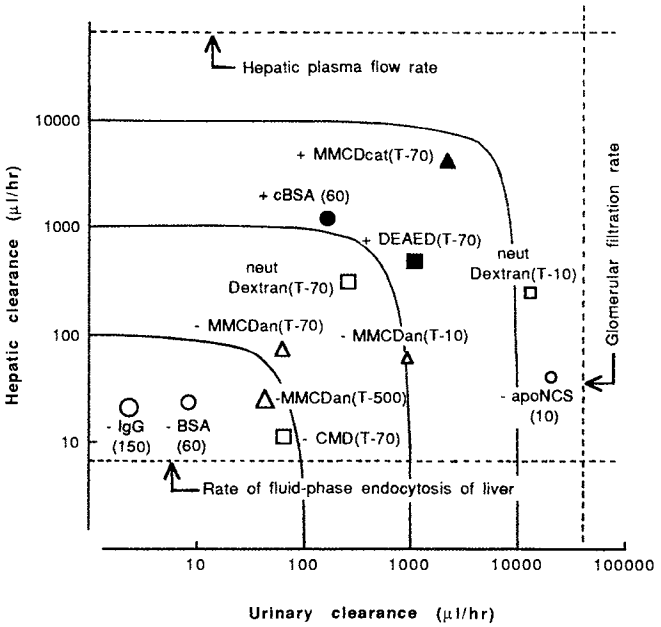

Fig. 6. Hepatic and urinary clearances of macromolecules in S180-bearing mice. Values for MMCDs were adopted from published data (14). Hepatic plasma flow rate (41), rate of fluid-phase endocytosis of liver (31), and glomerular filtration rate (34) were calculated assuming the weight of a mouse to be 25 g.

The present study revealed that tumor targeting could be achieved even with a target-nonspecific macromolecular carrier when appropriate physicochemical properties were selected. It was concluded that an anionic macromolecule with a high molecular weight would be advantageous as a carrier for passive targeting to the tumor.

ACKNOWLEDGMENTS

The authors wish to thank Drs. M. Sugiyama and T. Nakagawa, Nippon Shin'yaku, Co., Japan, for their helpful suggestions and technical assistance on the preparation of the radiolabeled dextrans. We also wish to thank Professor A. Yokoyama, Faculty of Pharmaceutical Sciences, Kyoto University, Japan, for his helpful suggestions for radiolabeling of proteins. A part of this work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan.

REFERENCES

1. E. Tomlinson. In E. Tomlinson and S. S. Davis (eds.), *Site-Specific Drug Delivery*, John Wiley & Sons, Chichester, 1986, pp. 1–26.
2. H. Sezaki, Y. Takakura, and M. Hashida. *Adv. Drug Deliv. Rev.* 3:247–266 (1989).
3. C.-W. Vogel. In C.-W. Vogel (ed.), *Immunoconjugates: Antibody Conjugates in Radioimaging and Therapy of Cancer*, Oxford University Press, New York, 1988, pp. 3–7.
4. H. Sezaki and M. Hashida. *CRC Crit. Rev. Ther. Drug Carrier Syst.* 1:1–38 (1984).
5. T. Kojima, M. Hashida, S. Muranishi, and H. Sezaki. *J. Pharm. Pharmacol.* 32:30–34 (1980).
6. M. Hashida, A. Kato, T. Kojima, S. Muranishi, H. Sezaki, N. Tanigawa, K. Satomura, and Y. Hikasa. *Gann* 72:226–234 (1981).
7. M. Hashida, Y. Takakura, S. Matsumoto, H. Sasaki, A. Kato, T. Kojima, S. Muranishi, and H. Sezaki. *Chem. Pharm. Bull.* 31:2055–2063 (1983).
8. M. Hashida, A. Kato, Y. Takakura, and H. Sezaki. *Drug Metab. Dispos.* 12:492–499 (1984).
9. Y. Takakura, S. Matsumoto, M. Hashida, and H. Sezaki. *Cancer Res.* 44:2505–2510 (1984).
10. S. Matsumoto, A. Yamamoto, Y. Takakura, H. Hashida, N. Tanigawa, and H. Sezaki. *Cancer Res.* 46:4463–4468 (1986).
11. Y. Takakura, K. Mori, H. Hashida, and H. Sezaki. *Chem. Pharm. Bull.* 34:1775–1783 (1986).
12. Y. Takakura, R. Atsumi, M. Hashida, and H. Sezaki. *Int. J. Pharm.* 37:145–154 (1987).
13. Y. Takakura, M. Kitajima, S. Matsumoto, M. Hashida, and H. Sezaki. *Int. J. Pharm.* 37:135–143 (1987).
14. Y. Takakura, A. Takagi, M. Hashida, and H. Sezaki. *Pharm. Res.* 4:293–300 (1987).
15. R. Atsumi, K. Endo, T. Kakautani, Y. Takakura, M. Hashida, and H. Sezaki. *Cancer Res.* 47:5546–5551 (1987).
16. S. Nakane, S. Matsumoto, Y. Takakura, M. Hashida, and H. Sezaki. *J. Pharm. Pharmacol.* 40:1–6 (1988).
17. K. Sato, K. Itakura, K. Nishida, Y. Takakura, M. Hashida, and H. Sezaki. *J. Pharm. Sci.* 78:11–16 (1989).
18. Y. Takakura, Y. Kaneko, T, Fujita, M. Hashida, H. Maeda, and H. Sezaki. *J. Pharm. Sci.* 78:117–121 (1989).
19. Y. Takakura, T. Fujita, M. Hashida, H. Maeda, and H. Sezaki. *J. Pharm. Sci.* 78:219–222 (1989).
20. T. Fujita, Y. Takakura, M. Hashida, and H. Sezaki. *J. Control. Release* (in press) (1989).
21. W. M. Mckerman and C. R. Rickettes. *Biochem. J.* 76:117–120 (1960).
22. E. A. Peterson and H. S. Sober. *J. Am. Chem. Soc.* 78:751–755 (1956).

23. W. M. Pardridge, A. K. Kumagai, and J. B. Eisenberg. *Biochem. Biophys. Res. Commun.* **146**:307–313 (1987).
24. A. K. Kumagai, J. B. Eisenberg, and W. M. Pardridge. *J. Biol. Chem.* **262**:15214–15219 (1987).
25. H. S. Isbel, H. L. Frush, and J. D. Moyer. *Tech. Assoc. Pulp Paper Ind.* **40**:739–742 (1957).
26. D. J. Hnatowich, W. W. Layne, and R. L. Childs. *Int. J. Appl. Isot.* **33**:327–332 (1982).
27. D. T. Mahin and R. T. Roftberg. *Anal. Biochem.* **16**:500–509 (1966).
28. R. W. Mowry and R. C. Millican. *Am. J. Pathol.* **29**:523–545 (1953).
29. R. G. Melton, C. N. Wiblin, A. Baskerville, R. L. Foster, and R. F. Sherwood. *Biochem. Pharmacol.* **36**:113–121 (1987).
30. B. A. Brown, R. D. Comeau, P. L. Jones, F. A. Liberatore, W. P. Neacy, H. Sands, and B. M. Gallagher. *Cancer Res.* **47**:1149–1154 (1987).
31. J. Munniksma, M. Noteborn, T. Kooistra, S. Steinstra, J. M. W. Bouma, M. Gruber, A. Brouwer, D. P. U. Dalen, and D. L. Knook, *Biochem. J.* **192**:613–621 (1980).
32. A. E. Taylor and N. D. Granger. In E. M. Renkin and C. C. Michel (eds), *Handbook of Physiology: The Cardiovascular System IV*, American Physiological Society, Bethesda, Md., 1984, pp. 467–520.
33. B. M. Brenner, T. H. Hostetter, and H. D. Humes. *Am. J. Physiol.* **234**:F455–F460 (1978).
34. R. L. Dedrick. *J. Pharmacokin. Biopharm.* **1**:435–461 (1973).
35. J. N. Purtell, A. J. Pesce, D H. Clyne, W. C. Miller, and V. E. Pollak. *Kid. Int.* **16**:366–376 (1979).
36. E. I. Christensen, H. G. Rennke, and F. A. Carone. *Am. J. Physiol.* **244**:F436–F441 (1983).
37. R. K. Jain. *Cancer Metas. Rev.* **6**:559–593 (1987).
38. C. W. Song and S. H. Levitt. *Cancer Res.* **31**:587–589 (1971).
39. S. W. O'Conor and W. F. Bale. *Cancer Res.* **44**:3719–3723 (1984).
40. D. Trigueo, J. B. Buciak, J. Yang, and W. M. Pardridge. *Proc. Natl. Acad. Sci.* **86**:4761–4765 (1989).
41. K. B. Bischoff, R. L. Dedrick, and D. S. Zaharko. *J. Pharm. Sci.* **59**:149–154 (1970).