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Body distribution of dextran derivatives with electric charges after intravenous administration

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Abstract

The body distribution of electrically charged dextran derivatives was investigated after intravenous administration of ^{125}I-labeled dextrans. The effects of the charge density and molecular weight of dextran derivatives on their half-lives in the circulation and organ distribution were pharmacokinetically analyzed. The introduction of negative charges into the dextran molecule prolonged its half-life in the circulation. This trend became more marked with increasing molecular weight of the dextran, however, its half-life was reduced by derivatization with cationic diethylaminoethyl groups. The cationized dextran accumulated in the liver more significantly than the anionized and original dextrans. For example, approx. 10% substitution of dextran with the diethylaminoethyl group was sufficient to enhance the accumulation of dextran in the liver and spleen, but no effect of cationic derivatization was observed for the distribution in other tissues.

Keywords: Dextran; Body distribution; Electric charge; Half-life; Liver targeting; Drug carrier

1. Introduction

Various water-soluble polymers possessing electrical charges have been employed as drug carriers, since their ionic features often provide useful effects that permit enhancement of the therapeutic efficacy of drugs modified with these polymers. For example, poly(L-lysine) (Shen and Ryser, 1979; Chu and Howell, 1982; Roos et al., 1984; Shen et al., 1989), poly(L-glutamic acid) (Roos et al., 1984), and poly(L-aspartic acid) (Zunino et al., 1982; Roos et al., 1984) have been investigated as carriers for antibiotics, such as mitomycin C and daunorubicin. Copolymers containing maleic anhydride as one monomer component were also studied as synthetic anionic carriers (Przybylski et al., 1978; Azori et al., 1986; Kobayashi et al., 1988; Han et al., 1990). Generally, anionic charges are reported to prolong the half-life of polymers in the blood circulation owing to their reduced interaction with tissues and cells, whereas cationic charges exhibit opposite behavior (Chu and Howell, 1982; Takakura et al., 1990). However, to our knowledge, no systematic research on the biological fate of dextrans with different molecular weights and electric charges

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has been reported as yet. To maximize the effectiveness of polymer carriers, information on the relationship between the physicochemical property of the polymers and their biological fate in the body is essential. In the present work, positively or negatively charged dextrans with different densities of electric groups and different molecular weights were synthesized and intravenously injected to mice. Their half-lives in the blood circulation and their organ distribution were investigated.

2. Materials and methods

2.1. Reagents

Dextrans with molecular weights of 28 000 and 53 500, and 197 000 were purchased from Sigma Chemical Co. (St. Louis, MO) and Pharmacia Biotech AB (Uppsala, Sweden), respectively. 2-Chlorotriethylamine (CTEA), 3-chloroethanesulfonic acid (CSA), and chloramine T were obtained from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Tyramine and sodium metabisulfite (SMS) were purchased from Nacalai Tesque (Kyoto, Japan). $Na^{125}I$ solution (740 MBq/ml) and Dowex® were obtained from NEN Research Products (Boston, MA) and Dow Chemical Co., Ltd (Midland, MI), respectively. Phosphate-buffered saline solution (PBS, pH 7.4) was obtained from Nissui Seiyaku Co., Ltd (Tokyo, Japan). Other chemical reagents were of guaranteed grade and used without further purification.

2.2. Animals

Specific pathogen-free inbred female BALB/cCrslc mice, 8–12 weeks old, were obtained from the Shizuoka Animal Facility Center (Shizuoka, Japan).

2.3. Preparation of sulfoethyl dextran and diethylaminoethyl dextran

A given amount of CTEA or CSA was added to 17.1 ml of 5 N NaOH solution containing 2 g of dextran with different molecular weights. After agitation at 80°C for 1–3 h in an atmosphere of nitrogen, the resulting mixture was dialyzed against double-distilled water using Spectrapor® dialysis membranes with a molecular weight cut-off of 1000 (Spectrum Medical Industries Inc., Los Angeles, CA) to remove unreacted reagents. The powdered form of sulfoethyl and diethylaminoethyl dextrans was obtained by lyophilization of the respective solution.

2.4. Determination of the substitution ratio of ionic groups in charged dextrans

The ratio of ionic groups introduced into the total hydroxyl groups of dextran was determined using the colloid titration method (Senju et al., 1969) and expressed as the percent substitution ratio (SR) of dextran. Briefly, 20 ml of 1 wt% aqueous solutions of sulfoethyl or diethylaminoethyl dextran containing 0.1 ml of toluidine blue solution as an indicator was titrated with N/200 methylglycol chitosan solution or N/400 potassium poly(vinyl sulfate) solution, respectively.

2.5. ^{125}I labeling of sulfoethyl and diethylaminoethyl dextrans

^{125}I labeling of sulfoethyl and diethylaminoethyl dextrans was carried out through radioiodination of tyramine residues introduced into hydroxyl groups of the respective dextran derivatives. Reaction of tyramine with hydroxyl groups of dextran was performed by use of *N,N'*-carbonyldiimidazole (CDI) (Beauchamp et al., 1983). 15 mg of CDI was added to 10 ml of 2.5% dextran solution in dimethyl sulfoxide, followed by addition of 120 mg of tyramine. The reaction was allowed to proceed at room temperature for 12 h under stirring to complete imidazolyl carbonylation of hydroxyl groups. The reaction mixture was then dialyzed against double-distilled water for 2 days using a Spectrapore® dialysis membrane to remove the unreacted reagent and lyophilized to obtain powdered tyramine-bound dextrans with negative or positive charges. The ratio of tyramine incorporation for all samples was adjusted to below 0.1% to minimize the effect on the body distribution pattern.

Radioiodination of the tyramine-bound charged dextrans was conducted with the chloramine T method (Greenwood et al., 1963). The charged dextran with tyramine residues was dissolved in 0.5 M phosphate buffer (KPB) (pH 7.5) to yield a concentration of 5 mg/ml. To 150 μl of the solution, 2 μl of $Na^{125}I$ solution and 100 μl of 0.2 mg/ml chloramine T in 0.05 M KPB (pH 7.2) were added. After agitating the solution for 2 min, 100 μl of PBS containing 4 mg/ml of SMS was added to stop the radioiodination. Then, the resulting mixture containing uncharged and positively charged dextrans was passed through the column of Dowex® to remove the uncoupled ^{125}I from the ^{125}I-labeled dextrans. In the case of negatively charged dextran, the uncoupled ^{125}I molecules in the reaction mixture were removed by a gel filtration method using a PD-10 packed column (Pharmacia Biotech AB, Uppsala, Sweden).

2.6. Determination of the blood concentration of dextran derivatives

0.1 ml of 0.1 wt% solutions of ^{125}I-labeled dextran derivatives with different substitution ratios in PBS was injected to mice via the tail vein. At different time intervals, blood samples of less than 50 μl were taken from the retroorbital plexus and their radioactivity was measured on a gamma counter (Autowell Gamma System Aloka ARC-301B, Aloka Co., Ltd, Tokyo, Japan). The percentage of radioactivity remaining in the whole blood was determined from the radioactivity concentration in the blood sample and the total volume of mouse blood. The latter was calculated from the dilution ratio of ^{125}I-labeled mouse serum albumin (MSA) under the assumption that most of the allogeneic MSA injected was present in the circulation without distributing to extravascular tissues at least during the early stage after intravenous (i.v.) injection. It has been demonstrated that allogeneic serum albumin injected intravenously shows a very long half-life and more than 95% of the injected dose remains in the blood concentration 30 min after injection (Poznansky and Bhardwaj, 1981). The radioactivity in the blood was measured employing 40 s as the sample time as short as possible after i.v. injection of ^{125}I-labeled MSA and compared with that of the initial ^{125}I-labeled MSA solution to estimate the dilution ratio of MSA. The frequency of successive blood sampling from one mouse was less than seven times to minimize the effect of decrease in blood volume on the plasma concentration of dextran. Blood sampling of six times had no effect on the decrement patterns of radioactivity from the blood circulation. Three mice were used for each experiment and each result is expressed as the mean ± S.D..

2.7. Analytical method of the blood concentration-time curve

The time profile of the blood concentration of electrically charged dextrans was analyzed using the two-compartment model. The time course of polymer concentration in the blood is described by Eq. 1 and the parameters A, B, α, and β in the equation were determined from experimental data by curve fitting using the non-linear least-squares program MULTI (Yamaoka et al., 1981):

$$C_{(t)} = A \cdot e^{-\alpha t} + B \cdot e^{-\beta t} \quad (1)$$

The half-life period in the circulation at the β phase, $T_{1/2,\beta}$, was calculated from the parameter β.

2.8. Determination of the body distribution of dextran derivatives

200 μl of the blood was directly collected using a heparinized syringe from the heart of mice which had received i.v. injection of ^{125}I-labeled dextrans. Then, the heart, lungs, thymus, liver, spleen, kidneys, gastrointestine (G.I.), and thyroid gland were excised from the mice, followed by rinsing twice with PBS. The radioactivity of the blood sample, each organ, and the carcass (the residual body parts involving muscle, skin, and bone) was measured on the gamma counter. The total radioactivity of excrements, urine, and feces was measured to assess the amount of polymer excreted.

It was too difficult to evaluate the real radioactivity accumulated in each organ, since the radioactivity measured involved what should be attributed to the blood remaining in the organ. Thus, to assess the net radioactivity distributed in the organ matrix, the blood volume of organ was evaluated from the total radioactivity of organs and the plasma concentration of radioactivity 40 s after i.v. injection of ^{125}I-labeled MSA. The real radioactivity of organs was estimated as the difference between the radioactivity of the whole organ and the blood in the organ. In the present study, the percentage of radioactivity recovered ranged from 90 to 110%.

2.9. Calculation of the clearance rate of dextran derivatives in each organ

The tissue distribution data were treated on the basis of a tissue uptake rate index (tissue clearance, CL_{in}) (Takakura et al., 1990) and can be expressed by Eq. 2:

$$CL_{in} = \frac{T_{(t_1)}}{\int_0^{t_1} C_{(t)}\,dt} = \frac{T_{(t_1)}}{AUC_{0-t_1}} \qquad (2)$$

where $T_{(t_1)}$ is the percentage of the amount accumulated in a tissue to that injected at time t_1 and AUC_{0-t_1} represents the area under the plasma concentration-time curve up to time t_1. In this equation, the efflux process of macromolecules from the tissues is neglected because they tend to be retained for a considerably long period following their tissue accumulation. However, in the case of very low molecular weight, the amount of dextran distributed in organs decreased with time probably due to their return to plasma from the tissue. It is, therefore, preferable to evaluate the organ clearance over a short time range after injection in order to minimize the efflux distribution. However, when the evaluation time was very short, it was difficult to accurately evaluate the organ accumulation of dextran. Thus, the organ distribution was measured at 10 min after i.v. injection of various dextran derivatives in the present experiment.

2.10. Interaction of the dextran derivatives with blood components

100 μl of 1% PBS solution containing ^{125}I-labeled dextran with different cationic charges was added to 200 μl of mouse blood and incubated for 10 min at 37°C. The mixture was applied on the density gradient centrifugation medium, Ficoll®, and centrifuged for 30 min at 2500 rpm. The radioactivity of each fraction of plasma, erythrocyte, and other cells collected was measured.

3. Results

3.1. Preparation of charged dextrans

Fig. 1 shows the effect of the concentration of CTEA and CSA added on the substitution ratio of charged residues to dextran. Apparently, the substitution ratio increased with an increase in the reagent concentration, for both reagents. However, the substitution ratio of diethylaminoethyl groups decreased when the CTEA concentration rose above 0.2 M. This is because a large amount of the reagent lowered the pH value of the reaction mixture, leading to a reduced rate of the substitution reaction. Therefore, to enhance the substitution ratio of diethylaminoethyl dextran, the substitution reaction was repeated several times using CTEA at a concentration less

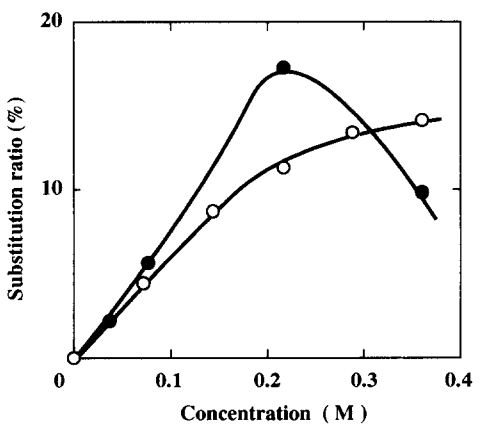

Fig. 1. Effect of the concentration of CES (○) and CTEA (●) on the substitution ratio of ionic groups into dextran.

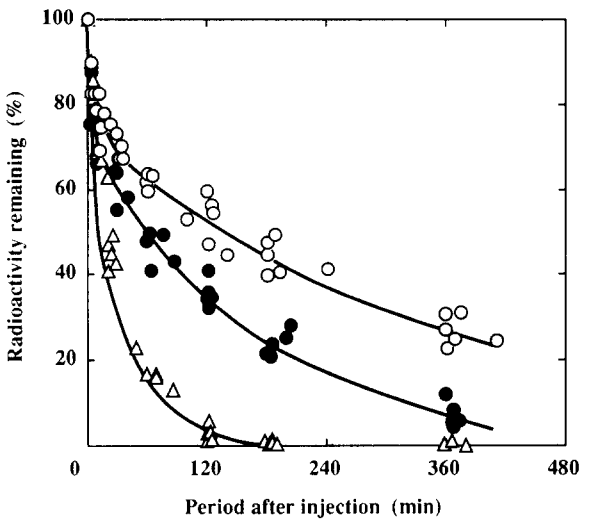

Fig. 2. Decrement patterns of sulfoethyl (○), diethylaminoethyl (△), and uncharged (●) dextrans administered intravenously in the blood circulation.

than 0.2 M. The change in the reagent concentration and reaction time enabled us to prepare dextran derivatives with different substitution ratios.

3.2. Plasma concentration of dextran derivatives administered intravenously

The decrement patterns of plasma concentration of ^{125}I-labeled dextran derivatives with a molecular weight of 197000 are illustrated in Fig. 2. The substitution ratio of diethylaminoethyl and sulfoethyl dextrans was 5.3 and 3.9%, respectively. The solid lines are curves calculated from the experimental data using the computer program and the calculated parameters are summarized in Table 1. The decrement pattern of the dextran derivatives was greatly influenced by their electric character. The positively charged dextran was eliminated rapidly from the blood circulation, while the negatively charged dextran remained in the circulation for a longer period than the uncharged dextran.

3.3. Half-life of dextran derivatives in the circulation

Fig. 3 shows the half-life of dextran derivatives in the blood circulation plotted as a function of

Table 1
Pharmacokinetic parameters of various dextran derivatives with a molecular weight of 197000

Parameter	Substituent		
	None	$N(C_2H_5)_2$ [a]	SO_3H [b]
A	18.1	25.6	18.4
α	0.334	0.357	0.220
B	34.4	29.4	35.0
β	0.00624	0.0245	0.00286

[a] SR = 5.3%; [b] SR = 3.9%.

their molecular weight. The substitution ratio of diethylaminoethyl groups was 3.8, 3.4, and 5.3% while that of sulfoethyl groups was 17.5, 4.9, and 3.9% for dextran molecular weights of 28000, 53500, and 197000. The half-life of dextrans was shortened by diethylaminoethylation but prolonged by sulfoethylation. Moreover, the derivatization effect on the half-life was dependent on the molecular weight of dextrans used. For the dextran with a molecular weight of 28000, derivatization had no effect on the half-life of dextran. However, this does not mean that derivatization had no effect on the body distribution of dextran. It is likely that cationization and anionization enable alteration of the distribution profile of dextran, leading to comparable half-lives. The half-life of uncharged dextran became longer with increasing molecular weight, when it was higher than 28000. The half-life of anionized dextran was prolonged with increasing molecular weight

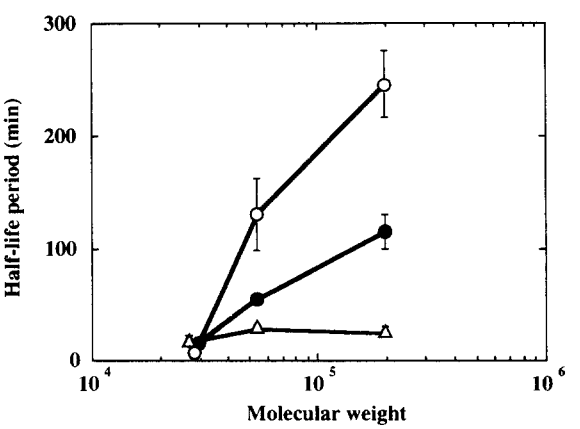

Fig. 3. Dependence of the half-life in the blood circulation on the molecular weight for sulfoethyl (○), diethylaminoethyl (△), and uncharged (●) dextrans.

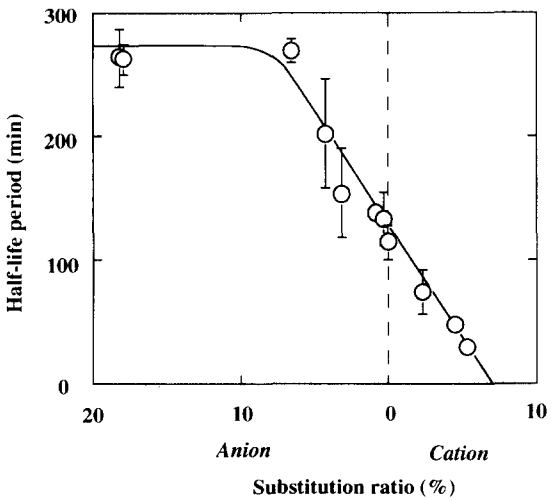

Fig. 4. Effect of the substitution ratio of charged groups on the plasma half-life of dextran derivatives with a molecular weight of 197000.

although that of cationized dextran was not changed.

Fig. 4 depicts the effect of electric charge on the half-life of ionic dextrans with a molecular weight of 197000 in the blood circulation. The half-life of dextran derivatives clearly depended on the density of electric charge. The higher the substitution ratio of sulfoethyl groups, the longer was the plasma half-life for substitution ratios less than 6.6%. However, substitution ratios higher than 6.6% did not lead to a longer half-life. In contrast, diethylaminoethylation markedly reduced the half-life of dextran. The data for highly cationized dextrans with substitution ratios of 9.8 and 17.4% are not plotted in Fig. 4, since their half-lives could not be measured as the mice given their injection died within 1 h after injection.

3.4. Organ distribution of dextran derivatives

Table 2 lists the organ distribution of dextran derivatives after i.v. injection. The percentage of dextrans remaining in the blood circulation was greatly affected by the type of charged group. The percentage remaining in the circulation 3 h after injection was 16, 0.6, and 41% for uncharged dextran, diethylaminoethyl dextran, and sulfoethyl dextran, respectively. Diethylaminoethyl dextran accumulated in every organ to a greater extent than the uncharged and sulfoethyl dextrans. A considerable amount of dextran derivatives accumulated in the liver and carcass, although the extent depended on the type of charged group. Approx. 4-times more diethylaminoethyl dextran accumulated in the liver than the uncharged dextran, while the extent of sulfoethyl dextran accumulation in the liver was about 25% less. A similar trend was observed for the spleen accumulation although the absolute

Table 2
Body distribution of ^{125}I-labeled dextran derivatives 1 and 3 h after intravenous injection

Organ	Percent distribution					
	No substituent		$-N(C_2H_5)_2$ [a]		$-SO_3H$ [b]	
	1 h	3 h	1 h	3 h	1 h	3 h
Blood	49.8 ± 5.1	16.3 ± 4.3	11.7 ± 4.2	0.6 ± 0.2	40.0 ± 0.2	40.9 ± 4.7
Heart	0.2 ± 0.1	0.1 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.2	0.1 ± 0.1
Lung	0.1 ± 0.1	0.3 ± 0.1	0.4 ± 0.0	0.5 ± 0.0	0.3 ± 0.0	0.3 ± 0.2
Liver	12.7 ± 0.9	15.1 ± 3.9	51.8 ± 3.6	50.8 ± 1.0	3.2 ± 0.2	3.9 ± 0.2
Spleen	1.0 ± 0.2	0.7 ± 0.2	3.4 ± 0.2	2.5 ± 0.1	0.1 ± 0.0	0.2 ± 0.0
Kidney	0.5 ± 0.1	0.6 ± 0.2	5.4 ± 0.9	4.7 ± 0.5	0.9 ± 0.2	0.9 ± 0.1
G.I.	2.8 ± 1.4	5.1 ± 3.2	6.4 ± 0.6	10.8 ± 0.3	2.5 ± 0.5	4.8 ± 1.1
Thyroid g and	0.4 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	1.0 ± 0.3	0.5 ± 0.1	3.0 ± 0.2
Carcass	4.1 ± 1.8	18.9 ± 4.1	9.2 ± 0.6	12.1 ± 0.5	5.5 ± 2.2	9.0 ± 2.6
Excrement	28.4 ± 1.7	40.5 ± 3.8	11.1 ± 0.4	16.4 ± 0.4	46.8 ± 2.6	36.8 ± 1.5
Recovery	88.5 ± 2.1	91.0 ± 8.6	79.0 ± 2.7	88.6 ± 2.0	85.7 ± 5.0	73.7 ± 3.1

[a] SR = 5.3%; [b] SR = 3.9%.
Mol. wt of dextran = 197000 (n = 3; mean ± S.D.).

Table 3
Organ clearances of various dextran derivatives

Organs	Clearance (μl/h per g)		
	No substituent	$N(C_2H_5)_2$ [a]	SO_3H [b]
Heart	55.6 ± 23.4	187.2 ± 98.6	36.2 ± 8.0
Lung	97.5 ± 45.4	145.4 ± 58.2	74.7 ± 30.1
Liver	304.6 ± 30.7	856.6 ± 113.2	53.6 ± 12.5
Spleen	365.3 ± 83.3	1349.6 ± 83.9	76.3 ± 27.8
Kidney	160.6 ± 82.0	203.2 ± 72.6	103.1 ± 81.2
G.I.	50.5 ± 26.9	39.8 ± 5.6	43.8 ± 4.3
Carcass	24.7 ± 9.2	27.7 ± 4.4	28.7 ± 5.4

[a] SR = 5.3%; [b] SR = 3.9%.
Mol. wt of dextran = 197000 (n = 3; mean ± S.D.).

percentage was much less than that of the liver. Moreover, the amount of excreted dextran was reduced by diethylaminoethylation. In addition, high accumulation of dextran derivatives was observed in the carcass, due to the large mass of the carcass (about 15 g/mouse). This factor can be normalized using clearance per g tissue and Table 3 shows that of the dextran derivatives. It is clear that the liver and spleen clearances of diethylaminoethyl dextran were much greater than the others. On the other hand, sulfoethyl dextrans show very small organ clearances in each organ and tissue.

Fig. 5 shows the effect of electric charge on the organ distribution of various dextran derivatives with a molecular weight of 197000 at 10 min after i.v. injection. The pattern of organ distribution of positively charged dextrans greatly depended on the substitution ratio, whereas no significant difference in distribution among the organs was observed for both the sulfoethyl and non-ionic dextrans within 10 min after injection. The positively charged dextran was accumulated in the liver to a great extent compared with the negatively charged and uncharged dextrans. When the substitution ratio was high, the cationized dextran distributed in the lung, kidney, spleen, and G.I. in addition to the liver. When the diethylaminoethyl dextran with the substitution ratios of 9.8 and 17.4 was intravenously injected, mice died within 1 h after injection. When organs were taken from mice which had received i.v. injection of highly cationized dextran, no hemorrhage was observed but many red spots were seen on the

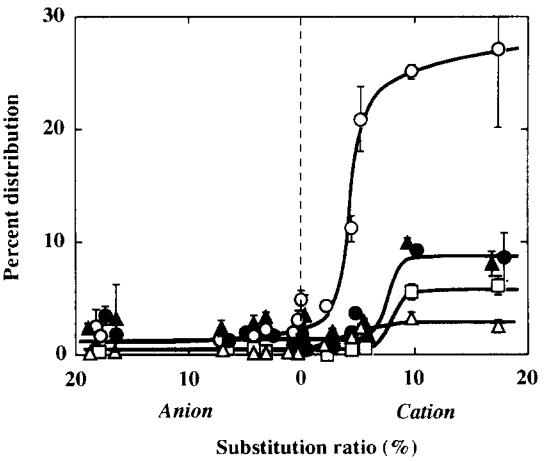

Fig. 5. Effect of the substitution ratio of charged groups on the percent distribution of dextran derivatives to the liver (○), spleen (△), lung (□), kidney (●), and G.I. (▲) 10 min after intravenous administration (n = 3).

lung surface. It is possible that blood coagulation was induced by the cationized dextran, leading to the death of mice. However, no blood coagulation was observed when the substitution ratio of cationic groups was as low as 4.5 and 5.3%.

3.5. Effect of the substitution ratio on the organ clearance of dextran derivatives

Fig. 6 shows the clearance of dextran derivatives with a molecular weight of 197000 per g of

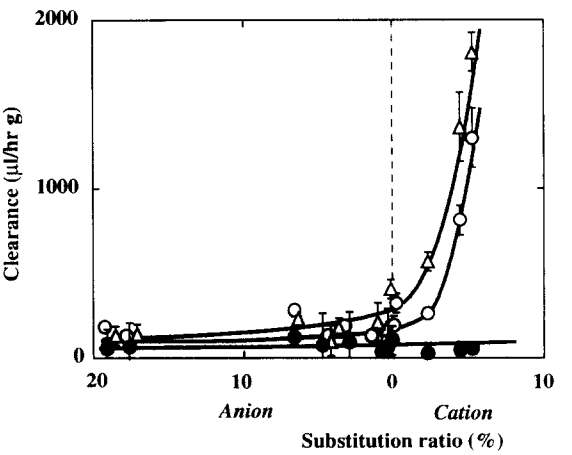

Fig. 6. Effect of the substitution ratio of charged groups on the clearance rate of dextran derivatives at the liver (○), spleen (△), and other organs (●) (n = 3).

the liver and spleen. The closed circles indicate the average value of the clearance rate in the organs and tissues other than the liver and spleen. Uncharged dextran was accumulated in the liver and spleen to a greater extent than in other tissues. Introduction of negative charges into dextran reduced the clearance of the liver and spleen but had no effect on the clearance of other tissues. On the other hand, cationization of dextran greatly affected the clearance and strongly enhanced the liver and spleen accumulation without any marked change in accumulation in other organs.

4. Discussion

The present study demonstrated that the biological fate of electrically charged dextrans was greatly influenced by the substitution ratio of charged groups and the molecular weight of dextran. It has been reported that, in general, anionization of dextran prolongs the half-life in the circulation, whereas cationization reduces the half-life (Shen and Ryser, 1979; Chu and Howell, 1982; Roos et al., 1984; Takakura et al., 1990). However, as shown in Fig. 3, it was found that the half-life of dextran derivatives in the circulation strongly depended not only on the electric charge type but also on the molecular weight. The ionization effect on the half-life was enhanced with increase in molecular weight. When the dextran of molecular weight 28000 was administered to mice, the half-life was around 10 min, irrespective of the electric charge. This may be explained in terms of the organ distribution of dextran derivatives. More than half of the injected uncharged dextran ($69.0 \pm 4.5\%$) was excreted within 1 h and the accumulated percentage in the liver and the other organs was 8.0 ± 0.7 and 23.0 ± 2.1, respectively. Dextran of low molecular weights may be readily excreted into the urine because of the high vascular permeability at the glomeruli. In contrast, the majority of injected diethylaminoethyl dextran was mainly detected in the liver ($39.5 \pm 1.3\%$) and other organs ($53.2 \pm 1.3\%$) but not in the excrement ($7.3 \pm 2.2\%$) 1 h after injection. On the other hand, only 2.4 ± 0.2 and $18.4 \pm 2.6\%$ of total sulfoethyl dextran distributed in the liver and the other organs, respectively, and $79.2 \pm 7.8\%$ of the total dose was detected in the excrement. It seems probable that the short half-lives of cationic and anionic or uncharged dextran will be ascribed to different reasons. The former tended to be readily accumulated into organs especially liver while the latter tended to be excreted into the urine. It is likely that every dextran derivative is rapidly eliminated from the blood circulation, leading to a similar short half-life although their profile of body distribution is different.

Introduction of sulfoethyl groups into dextran with higher molecular weights significantly prolonged the half-life, whereas introduction of diethylaminoethyl groups reduced the half-life of dextran. For the case of a high molecular weight of dextran, the electric charge will affect the half-life of dextran derivatives and the longer half-lives of negatively charged dextrans than the uncharged dextran may be due to the reduced organ clearances (Table 3). On the other hand, positively charged dextrans that undergo a strong interaction with tissues and organs showed very short half-lives in the circulation because of large organ clearances (Fig. 6 and Table 3).

The half-life of anionic dextran in the blood circulation was prolonged with increase in the substitution ratio over the range less than 6.6%. However, substitution ratios higher than 6.6% did not prolong the half-life period any further. As shown in Fig. 5, anionic dextran was scarcely accumulated in organs, leading to a high concentration in the circulation for a long period. This prolonged circulation of anionic dextran will lead to high plasma concentrations and enhancement of the amount excreted (Table 2). Hashida et al. (personal communication) found that carboxymethyl dextran was hardly accumulated in the liver, while dextran sulfate was located rapidly there. This suggests that the type of anionic group also affects their biological fate, often changing the profile of organ distribution.

The half-life of cationic dextrans in the circulation became shorter with increase in the substitution ratio of positively charged groups. Fig. 6 demonstrates that the cationic dextrans were pre-

dominantly accumulated in the liver and spleen at a similar clearance value. Nakane et al. (1987) reported high accumulation of cationic dextrans in the liver, especially in the parenchymal cells, while other research groups found high biliary excretion of ionized dextrans (Kagawa and Tomizawa, 1980; Hashida et al., 1990). These findings suggest that diethylaminoethyl dextran is effective as a water-soluble carrier to target drugs to the liver and spleen. However, an increase in the substitution ratio of diethylaminoethyl groups gave rise to death of mice under the present experimental conditions. This may be because highly cationized dextrans also interact strongly with the blood cells. Indeed, an in vitro experiment revealed that highly cationized dextrans distributed to cell fractions twice as much as that of dextran with low cationization extents. This indicates that highly cationized dextrans interacting with blood cells induce blood coagulation, which is fatal to life.

In conclusion, the diethylaminoethyl dextran with the substitution ratio about 5% will be useful as drug carrier for targeting drugs to the liver, while sulfoethylation prolongs the half-life of dextran, although their toxicity should be examined in detail in the future.

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