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# Absorption Characteristics of Macromolecular Prodrugs of Mitomycin C Following Intramuscular Administration

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The absorption characteristics of several kinds of macromolecular compounds following intramuscular injection were studied in rats. Three types of $^{14}C$-labeled mitomycin C–dextran conjugates ($^{14}C$-MMC-D), bare $^{14}C$-dextran, $^{14}C$-inulin, and $^{131}I$-albumin were used, and their absorption and subsequent lymphatic uptake were determined by radioactivity counting. Clearance of cationic $^{14}C$-MMC-D from the injection site was slow and varied with the molecular weight. Remarkable accumulation of radioactivity was observed in the regional lymph node after injection of $^{14}C$-MMC-D. Bare $^{14}C$-dextran showed similar behavior, but the rate of clearance was faster and less accumulation in the lymph node was observed compared with $^{14}C$-MMC-D of the same molecular weight. In contrast, $^{14}C$-inulin and $^{131}I$-albumin showed rapid disappearance from the muscle and no accumulation in the lymph node. Muscular absorption data were fitted to a compartment model including a Langmuir-type adsorption. Pharmacokinetic analysis revealed that the rate of absorption and the degree of adsorption depended on the molecular weight and electric charge, and MMC-D with a molecular weight of 500000 proved to have the longest period of residence. Thus, it was elucidated that physicochemical properties, such as molecular weight and electric charge, affect the pharmacokinetic behavior of macromolecular compounds injected topically.

**Keywords**——macromolecular compound; mitomycin C–dextran conjugate; dextran; inulin; albumin; intramuscular injection; absorption characteristic; lymphatic transfer; radioactivity counting; pharmacokinetic analysis

In cancer chemotherapy, it is very important to control the pharmacokinetic behavior of the anticancer agents since most of them have indiscriminate cytotoxicity toward both malignant and normal cells. A great deal of research effort has been expended to find ways of optimizing anticancer drug delivery.[1] In our laboratory, several drug delivery systems for anticancer agents have been developed from the viewpoints of utilizing physical devices,[2,3] chemical transformation of the drug molecules,[4,5] and combinations of physical and chemical approaches.[6]

Among these approaches, application of macromolecular compounds as carriers for antineoplastic agents seems to be promising since molecular size is one of the dominant determinants of the pharmacokinetic fate of a drug.[7] In the literature, various kinds of macromolecules[8-12] have been utilized and evaluated as candidates for tumor-specific delivery devices. In spite of numerous reports on the pharmacological efficacies of the polymer–drug conjugates, however, little is known about their *in vivo* disposition characteristics and pharmacokinetics, especially in the case of local administration.[7]

In our series of investigations, we have developed a polymeric prodrug of mitomycin C (MMC), MMC–dextran conjugate (MMC-D), by coupling MMC to dextran with the use of ε-aminocaproic acid as a spacer.[13] MMC-D exhibited superior activities against various murine tumors as compared with MMC,[14] and the size of the carrier dextran was found to

affect the efficiency.[15] Pharmacokinetic studies after systemic administration revealed that MMC-D acts as a reservoir which behaves in accordance with its macromolecular nature, while supplying active MMC in the body by chemical hydrolysis.[16] In addition, intramuscular (*i.m.*) injection resulted in retardation of the clearance from the injection site and enhancement of the lymphotropicity of MMC to a great extent.[17] Successful prevention of lymph node metastasis by MMC-D injection in mice suggested the therapeutic utility of this compound, and in a clinical trial involving intratumoral administration of MMC-D remarkable reduction in tumor size was obtained.[18]

The purpose of the present study was to clarify the pharmacokinetic character of the muscular absorption and subsequent lymphatic transfer of MMC-D using $^{14}C$-labeling at the position of the spacer. A comparative study was carried out with regard to bare dextran, inulin, and albumin. The absorption of macromolecules was characterized by pharmacokinetic analysis assuming a Langmuir-type adsorption step in the muscle.

## Experimental

**Material**——MMC was kindly supplied by Kyowa Hakko Co., Tokyo. Dextrans with various molecular weights were purchased from Pharmacia, Uppsala, Sweden, and had average molecular weights of about 10000 (T-10), 70000 (T-70), and 500000 (T-500). [$^{14}$C(U)] $\gamma$-Aminobutyric acid (2 mCi/mg), [carboxyl-$^{14}$C]dextran (1 $\mu$Ci/mg, $M_r$: 70000), and [carboxyl-$^{14}$C]inulin (42 $\mu$Ci/mg, $M_r$: 5000) were obtained commercially from New England Nuclear, Boston, MA. [$^{131}$I]Human serum albumin was purchased from Daiichi Radioisotopes, Tokyo. All other chemicals were commercial reagent grade products. Radiolabeled MMC-D was synthesized as reported previously.[13] Dextran was activated with cyanogen bromide at pH 10.7 and $\varepsilon$-aminocaproic acid was coupled to a glucose chain as a spacer. Radiolabeled $\gamma$- aminobutyric acid was introduced onto dextran together with $\varepsilon$-aminocaproic acid at a molar ratio of 1 : 1000. MMC was conjugated to the spacer-carring dextran by the use of a carbodiimide. All conjugates were estimated to contain MMC to almost equal extents of about 10% (w/w). The purified radiolabeled MMC-D has a specific activity of 0.05 $\mu$Ci/mg. MMC-D for injection was prepared in saline solution at a concentration of 2 mg eq MMC/ml. All other macromolecular compounds were dissolved in saline at a concentration of 20 mg/ml for experiments.

**Muscular Absorption Studies**——Male Wistar albino rats were obtained from Shizuoka Agricultural Cooperative Association for Laboratory Animals, Shizuoka. The rats weighing between 220 and 250 g were anesthetized with ether and injected with 100-$\mu$l aliquots of saline solution of the test compounds into the center of the left thigh muscle using a microsyringe. At various periods after the injection, rats were anesthetized with ether, and the thigh muscle and regional lymph node (left iliac node)[19] were excised. A blood sample was obtained simultaneously from the aorta using a heparinized syringe. Blood was centrifuged at 3000 rpm to obtain a plasma sample.

**Thoracic Lymph Collection**——Rats were anesthetized with sodium pentobarbital, and the thoracic duct was cannulated according to the method of Bollman *et al.*[20] A heparinized flexible vinyl catheter (Dural Plastics, Dural, Australia; i.d. 0.8 mm, o.d. 1.2 mm) was inserted into the thoracic duct and fixed with the aid of surgical adhesive (Sankyo Co., Tokyo). Following surgery, rats were kept in restraining cages with free intake of food and water. These procedures allowed continuous collection of lymph for 48 h. Injections were carried out at 30 min after surgery.

**Analytical Method**——The procedure for the determination of $^{14}C$ radioactivity was a modification of the method of Mahin and Loftberg.[21] The thigh muscle was solubilized in 1 N NaOH solution by shaking overnight at 37 °C and diluted to an appropriate volume with the same medium. Then, 0.2 ml of sample solution was put into a counting vial, and equal volumes of perchloric acid (60%) and hydrogen peroxide (35%) were added. The resulting mixture was heated at 70 °C for 90 min with agitation. After cooling to room temperature, 10 ml of scintillation

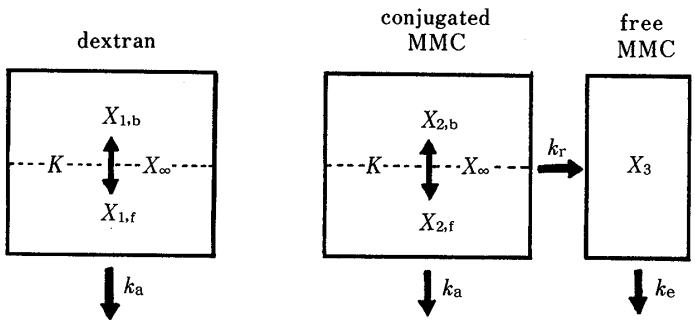


Fig. 1. Pharmacokinetic Model Describing the Disappearance of MMC-D from the Injection Site after Intramuscular Injection

medium (Univer-gel; Nakarai Chemicals, Kyoto) was added and the radioactivity was determined in a liquid scintillation system. The lymph nodes, plasma, and thoracic lymph were assayed without solubilization with NaOH. The counts obtained were corrected using external standards. The $^{13}$I-radioactivities in the muscle, lymph node, plasma, and thoracic lymph were determined with a well NaI-scintillation counter without any special procedure.

**Pharmacokinetic Analysis**——The data on disappearance from the injection site were analyzed by an iterative nonlinear least-squares method using a personal computer (PC 9801, NEC, Tokyo). The pharmacokinetic compartment model shown in Fig. 1 was used to describe the disposition of MMC-D after *i.m.* injection. Several assumptions were made in this model. 1) MMC-D is bound to the thigh muscle according to a Langmuir-type adsorption. 2) The binding parameters do not change during the period studied. 3) Unbound MMC-D is absorbed from the muscle according to first-order kinetics, but bound MMC-D is not. 4) The conversion of MMC-D to MMC proceeds at the same rate whether MMC-D is bound or unbound, and direct inactivation of MMC in the form of the conjugate does not occur. The following differential equations based on the model in Fig. 1 predict the amount of carrier dextran of MMC-D ($X_1$), conjugated MMC in MMC-D ($X_2$) and regenerated free MMC ($X_3$) as a function of time after *i.m.* injection of MMC-D with various molecular weights.

$$dX_1/dt = -k_a X_{1,f} \tag{1}$$

$$\begin{aligned} dX_2/dt &= -k_a X_{2,f} - k_r X_2 \\ &= -k_a X_{1,f} X_2/X_1 - k_r X_2 \end{aligned} \tag{2}$$

$$dX_3/dt = k_r X_2 - k_e X_3 \tag{3}$$

where $X_{1,f}$ and $X_{2,f}$ are the amounts of unbound carrier dextran and conjugated MMC, and $k_a$, $k_r$, and $k_e$ are the absorption rate constant of MMC-D, the regeneration rate constant from MMC-D to MMC, and the elimination rate constant of free MMC, respectively. The total amount of carrier dextran ($X_1$) can be described by a Langmuir-type equation:

$$\begin{aligned} X_1 &= X_{1,f} + X_{1,b} \\ &= X_{1,f} + X_\infty K X_{1,f}/(1 + K X_{1,f}) \end{aligned} \tag{4}$$

where $X_{1,b}$, $K$, and $X_\infty$ are the amount of bound $^{14}$C-dextran, the binding constant, and the maximum amount of $^{14}$C-dextran that can be adsorbed on the muscle, respectively. The quadratic from Eq. 4 gives

$$X_{1,f} = 1/2\{X_1 - X_\infty - 1/K + \sqrt{(X_\infty + 1/K - X_1)^2 + 4X_1/K}\} \tag{5}$$

Substituting Eq. 5 into Eqs. 1 and 2 gives

$$dX_1/dt = -k_a/2\{X_1 - X_\infty - 1/K + \sqrt{(X_\infty + 1/K - X_1)^2 + 4X_1/K}\} \tag{6}$$

$$dX_2/dt = -k_a/2\{X_1 - X_\infty - 1/K + \sqrt{(X_\infty + 1/K - X_1)^2 + 4X_1/K}\} X_2/X_1 - k_r X_2 \tag{7}$$

The muscle clearance data of carrier dextran shown in Fig. 2 and those of the conjugated form of MMC (published data)[17] were fitted simultaneously to Eqs. 6 and 7, respectively, and the parameters, $k_a$, $X_\infty$, $K$, and $k_r$ were determined by using the nonlinear least-squares program MULTI (RUNGE).[22] The interval for numerical integration of the differential equation was 0.1 h and each datum was weighted by the reciprocal of its square. For bare dextran and albumin, parameters were computed using Eq. 6, and the data for inulin were analyzed according to the simple first-order elimination kinetics without an adsorption step.

## Results

### Disappearance from the Injection Site

Figure 2 shows the disappearance of radioactivity from the thigh muscle after *i.m.* injection of $^{14}$C-MMC-D with various molecular weights (A) or radiolabeled dextran ($M_r$: 70000), albumin, or inulin (B). Since the $^{14}$C-label was confirmed to be stable in tissue for more than 48 h,[16] the $^{14}$C radioactivity of MMC-D was considered to show the pharmacokinetic behavior of the carrier dextran. Carrier dextran exhibited relatively slow clearance from the injection site, and the extent of elimination appeared to depend on the molecular weight. The disappearance curves were biphasic and the radioactivities showed an almost constant level during the second 24 h after administration. Approximately 23, 33, and 41% of the dose still remained in the thigh muscle even at 48 h after injection in the cases of MMC-D (T-10), MMC-D (T-70), and MMC-D (T-500), respectively. Bare $^{14}$C-dextran also exhibited a

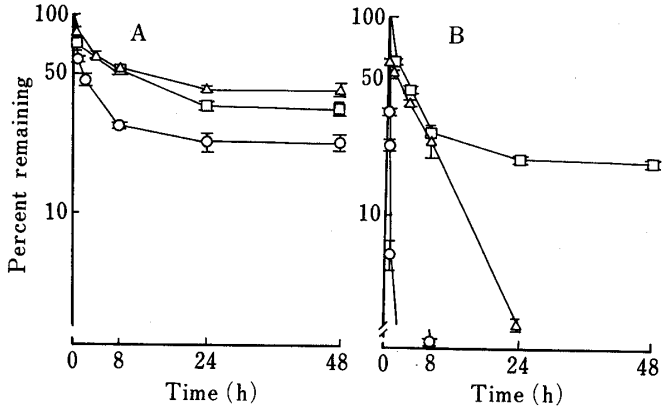


Fig. 2. Disappearance of Radioactivity from the Thigh Muscle after Intramuscular Injection of $^{14}$C-MMC-D (A) and Radiolabeled Macromolecules (B)

A: ○, MMC-D (T-10); □, MMC-D (T-70); △, MMC-D (T-500). B: □, $^{14}$C-dextran; ○, $^{14}$C-inulin; △, $^{131}$I-albumin. Each result is the mean±S.E. of at least four rats.

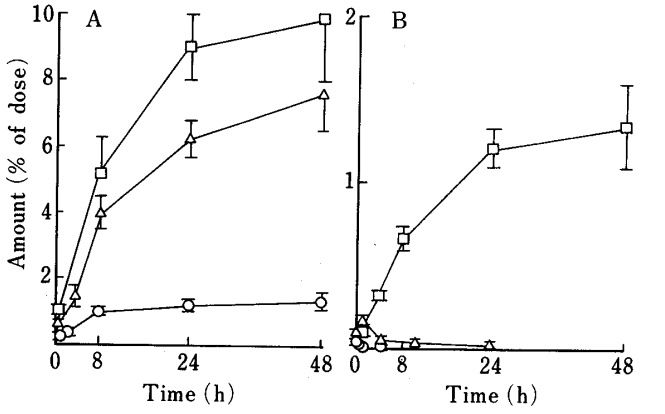


Fig. 3. Amount of Radioactivity in the Iliac Lymph Node after Intramuscular Injection of $^{14}$C-MMC-D (A) and Radiolabeled Macromolecules (B)

A: ○, MMC-D (T-10); □, MMC-D (T-70); △, MMC-D (T-500). B: □, $^{14}$C-dextran; ○, $^{14}$C-inulin; △, $^{131}$I-albumin. Each result is the mean±S.E. of at least four rats.

relatively slow and biphasic disappearance pattern, and 20% of the dose was still present at 48 h. On the other hand, the disappearance patterns of $^{14}$C-inulin and $^{131}$I-albumin were almost monoexponential and inulin showed the most rapid clearance.

**Regional Lymph Node Uptake**

Figure 3 illustrates the time course of radioactivity in the iliac lymph node following the *i.m.* injection of $^{14}$C-MMC-D (A), or $^{14}$C-dextran, $^{14}$C-inulin, or $^{131}$I-albumin (B). Radioactivity was accumulated in the lymph node after the injection of all types of $^{14}$C-MMC-D. In particular, MMC-D (T-70) showed a remarkable accumulation in the lymph node. Although a similar tendency was observed in the case of bare $^{14}$C-dextran, the total activity was only one-seventh of that of MMC-D (T-70). On the other hand, $^{14}$C-inulin and $^{131}$I-albumin gave trace levels of radioactivity in the regional lymph node.

**Transfer to the Thoracic Lymph**

Figure 4 shows the cumulative amount of radioactivity recovered in the thoracic lymph following *i.m.* injection of $^{14}$C-MMC-D (A), and other macromolecules (B). Only a small amount was transferred to the thoracic lymph in the case of MMC-D. The recovery decreased with increase of molecular weight, that is, 1.8, 0.4, and 0.2% of the dose were recovered within 48 h after injection of MMC-D (T-10), MMC-D (T-70), and MMC-D (T-500), respectively. On the other hand, $^{14}$C-dextran and $^{131}$I-albumin transferred well to the thoracic lymph, and the total recovery was approximately 9% of the dose in both cases. Rapid appearance of radioactivity was observed after dosing of $^{14}$C-inulin, and 1.4% of the dose was recovered in the lymph fluid within 8 h.

**Plasma Concentration**

Figure 5 shows the plasma concentration time courses of $^{14}$C-MMC-D (A), and three

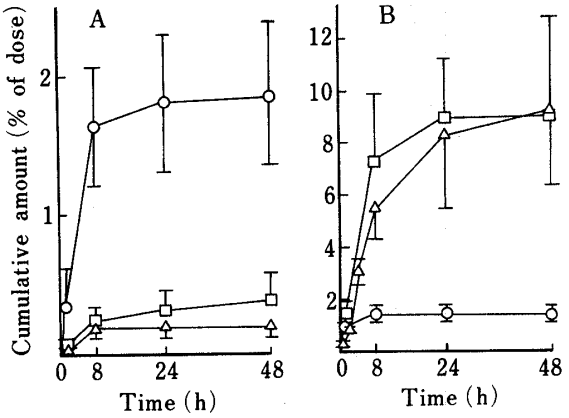


Fig. 4. Cumulative Amount of Radioactivity Transferred to the Thoracic Lymph after Intramuscular Injection of $^{14}$C-MMC-D (A) and Radiolabeled Macromolecules (B)

A: ○, MMC-D (T-10); □, MMC-D (T-70); △, MMC-D (T-500). B: □, $^{14}$C-dextran; ○, $^{14}$C-inulin; △, $^{131}$I-albumin. Each result is the mean±S.E. of at least four rats.

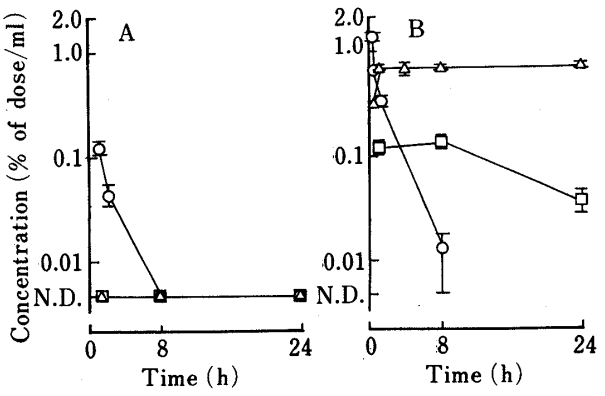


Fig. 5. Plasma Concentration of Radioactivity after Intramuscular Injection of $^{14}$C-MMC-D (A) and Radiolabeled Macromolecules (B)

A: ○, MMC-D (T-10); □, MMC-D (T-70); △, MMC-D (T-500). B: □, $^{14}$C-dextran; ○, $^{14}$C-inulin; △, $^{131}$I-albumin. Each result is the mean±S.E. of at least four rats. N.D., not detected.

TABLE I. MMC/Dextran Ratio in the Thigh Muscle and Iliac Lymph Node

| Tissue | Compound | 1 h | 2 h | 4 h | 8 h | 24 h | 48 h |
|---|---|---|---|---|---|---|---|
| Muscle | MMC-D (T-10) | 0.879 | 0.875 | — | 0.790 | 0.584 | 0.237 |
| | MMC-D (T-70) | 0.944 | — | — | 0.575 | 0.608 | 0.310 |
| | MMC-D (T-500) | 0.961 | — | 0.847 | 0.863 | 0.655 | 0.374 |
| Lymph node | MMC-D (T-10) | 0.544 | 0.270 | — | 0.151 | 0.106 | 0.035 |
| | MMC-D (T-70) | 0.378 | — | — | 0.068 | 0.032 | 0.007 |
| | MMC-D (T-500) | 0.510 | — | — | 0.088 | 0.038 | 0.019 |

Data for MMC have been published[17] and those for dextran are taken from Fig. 1(A) and Fig. 2(A).

kinds of radiolabeled macromolecules (B) after *i.m.* injection. Radioactivity was not detected in plasma following the administration of $^{14}$C-MMC-D at any sampling period, except for $^{14}$C-MMC-D (T-10). On the other hand, bare $^{14}$C-dextran maintained a relatively high concentration up to 24 h after injection. A consistent and high plasma level was obtained by administration of $^{131}$I-albumin, whereas $^{14}$C-inulin appeared immediately in the plasma, followed by a rapid decline.

**Stability of MMC Conjugated with Dextran in Muscle and Lymph Node**

In a previous investigation,[17] we periodically determined the amount of MMC (% of dose) in the muscle and lymph node after *i.m.* injection in the form of dextran conjugate. To clarify the disposition of MMC-D, therefore, the ratios of amount of conjugated MMC determined by bioassay[17] to that of $^{14}$C-MMC-D at each sampling time in the muscle and lymph node were calculated, and the results are summarized in Table I. Regardless of the molecular weight of carrier dextran, the ratio in the muscle decreased gradually and the ratios remained between 0.24 and 0.37 at 48 h after injection. On the other hand, a rapid decrease of MMC/dextran ratio was observed in the lymph node after injection of all types of MMC-D.

**Pharmacokinetic Analysis of Muscular Absorption**

The computer-generated parameters for the three types of MMC-D are summarized in

TABLE II. Computer-Estimated Pharmacokinetic Parameters for Disappearance from the Injection Site after Intramuscular Injection of MMC-D, Dextran, Inulin, and Albumin

| Parameter[a] | MMC-D | | | Dextran | Inulin | Albumin |
|---|---|---|---|---|---|---|
| | T-10 | T-70 | T-500 | | | |
| $k_a$ ($h^{-1}$) | 0.878 | 0.702 | 0.395 | 0.776 | 3.68 | 3.66 |
| $X_\infty$ (% of dose) | 29.4 | 42.7 | 49.8 | 25.9 | — | 59.5 |
| $K$ ((% of dose)$^{-1}$) | 30.4 | 22.0 | 16.1 | 20.7 | — | 1.55 |
| $k_r$ ($h^{-1}$) | 0.0285 | 0.0247 | 0.0198 | — | — | — |

a) $k_a$, absorption rate constant; $X_\infty$, the maximum amount that can be adsorbed by the muscle; $K$, binding constant; $k_r$, regeneration rate constant from MMC-D to MMC.

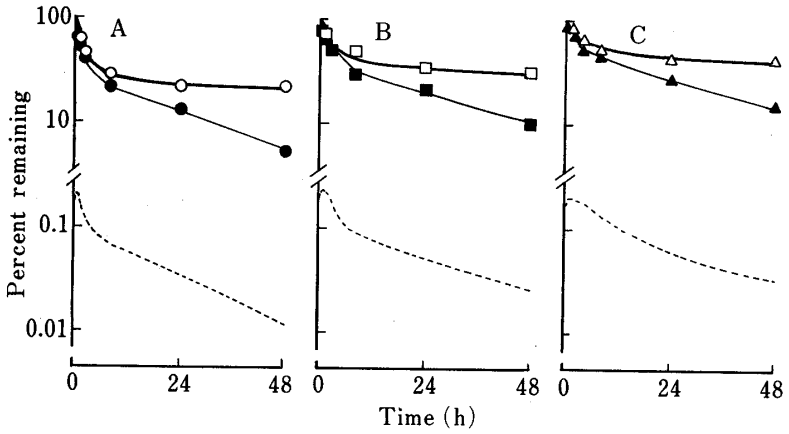


Fig. 6. Computer-Generated Time Courses of Carrier Dextran (——), Conjugated MMC (——), and Free MMC (------) at the Injection Site after Intramuscular Injection of MMC-D (T-10) (A), MMC-D (T-70) (B), and MMC-D (T-500) (C)

The theoretical curves were calculated based on the equation (1)—(3) (see text) using the parameters given in Table II. ○, □, △, observed values of carrier dextran; ●, ■, ▲, observed values of conjugated MMC.

Table II together with those for bare dextran, inulin, and albumin. MMC-D exhibited similar conversion rates ($k_r$), regardless of their molecular weights, and these rates showed good coincidence with the *in vitro* hydrolysis rate of MMC-D (0.029 $h^{-1}$) at pH 7.4 obtained previously.[23] On the other hand, the absorption rate ($k_a$) and Langmuir constant ($X_\infty$) varied with the molecular weight. Figure 6 shows the computer-generated curves for the amounts of carrier dextran and conjugated MMC. As shown in this figure, these curves describe the data rather well. The amount of free MMC was calculated based on Eq. 3 using the elimination constant of free MMC ($k_e = 9.20\,h^{-1}$) determined in the previous absorption study of free MMC,[17] and is also shown in Fig. 6.

## Discussion

Our recent study demonstrated the utility of MMC-D in local injection therapy based on the disposition characteristics of this compound.[17,18] In the present paper, the fate of carrier dextran and conjugated MMC were examined in order to clarify the disposition and the pharmacokinetics of MMC-D after local administration. In addition, the relationship between the fate of macromolecules and their physicochemical properties such as molecular weight and electric charge was investigated to obtain information usefull for macromolecular

prodrug design. The consecutive transfer of macromolecules between four compartments, that is, the injection site, regional lymph node, thoracic lymph, and central circulation was determined by radioactivity counting for accurate evaluation.

Sund and Schou[24] reported that the absorption rates of intramuscularly injected compounds were inversely related to molecular weight, and a significant difference was observed between $^{14}$C-inulin ($M_r$: 3000—4000) and $^{14}$C-dextran ($M_r$: 60000—90000) for the initial 1 h, which is also in good accordance with our results, as shown in Fig. 1(B). The effect of molecular weight on the disappearance was clear for the carrier dextran following the injection of $^{14}$C-MMC-D (Fig. 1(A)). However, the disappearance of MMC-D (T-70) was slower than that of bare dextran ($M_r$: 70000) and much slower than that of albumin ($M_r$: 66000). Thus these macromolecular compounds showed different disappearance rates in spite of having almost the same molecular weight. This might be caused by the difference in the electric charge, *i.e.*, MMC-D tested in the present investigation has a cationic charge in a neutral pH range[23] while bare dextran is neutral and albumin has an anionic charge (pI $\fallingdotseq$ 5). Thus, electric charge seems to play an important role in muscular absorption, besides molecular weight.

Macromolecules are generally known to be absorbed mainly by the lymphatic pathway but not by the capillary route from the interstitial space.[25] The results shown in Figs. 3—5, suggested that the main absorption route of MMC-D from the muscle is also the lymphatics. Carrier dextran was accumulated in the regional lymph node after *i.m.* injection of $^{14}$C-MMC-D. $^{14}$C-MMC-D (T-70) and $^{14}$C-MMC-D (T-500) exhibited outstanding accumulation, while transfer to the thoracic lymph was restricted. Thus, larger MMC-D was accumulated in the lymph node to a great extent, but did not flow into the thoracic lymph fluid. On the other hand, the transfer pattern of $^{14}$C-MMC-D (T-10) suggested that this compound was taken up by the lymphatic vessels, passed through the lymph node, and appeared in the thoracic lymph. The original $^{14}$C-dextran also appeared to be absorbed by the lymphatics. However, $^{14}$C-dextran exhibited a weaker affinity to the lymph node and a higher transferability to thoracic lymph, compared with MMC-D (T-70), which has the same molecular weight, apparently owing to the difference in electric charge. In contrast, it is likely that inulin and albumin were cleared by the capillary route, because only a trace amount was detected in the lymph node in each case. Inulin was readily absorbed by the capillary route and was subsequently cleared from plasma, possibly by rapid urinary excretion. Although a considerable amount of albumin was recovered in the thoracic lymph fluid, this could be explained by indirect transfer *via* the central circulation.

MMC-D thus proved to have a strong affinity for the muscle and the lymph node. Although the mechanism of interaction of MMC-D with these tissues is not clear, it is clear that the cationic charge of MMC-D is one of the most important factors. MMC-D might be adsorbed on these tissues by electrostatic forces. Our *in vitro* study revealed that MMC-D was strongly adsorbed on tumor cell surfaces with negative charge, and the adsorption could be well explained in terms of Langmuir-type adsorption.[26] Based on these observations, the compartment model with Langmuir-type adsorption (Fig. 1) was proposed for describing the absorption of MMC-D and other macromolecules from the injection site. Absorption was assumed to be first-order, since diffusion is the main driving force for the absorption of water-soluble compounds.[24] Several additional compartment models were derived and tested for ability to describe the sets of data for all MMC-D and the data for other macromolecular compounds. The general two-compartment model could also describe the biphasic disappearance of MMC-D, dextran, and albumin to some extent. However, only the present model could describe the plateau phase of carrier dextran elimination and showed an excellent fit on the basis of Akaike's information criterion[27] for all types of MMC-D. The residence time in the muscle can be evaluated from the binding parameters ($X_\infty$, $k$) as shown in Table II, and

MMC-D (T-500) was proved to have the best binding ability among the macromolecular compounds tested in this study.

Table I shows that MMC-D was stable and only the hydrolysis of MMC-D to MMC proceeded in the muscle; this is consistent with the results of an *in vitro* stability experiment using muscle homogenate.[23] On the other hand, the MMC/dextran ratio decreased rapidly in the lymph node. MMC-D seemed to undergo a rapid inactivation in the lymph node. MMC-D seemed to undergo a rapid inactivation in the lymph node, since liver tissue homogenate or plasma did not accelerate the hydrolysis of MMC-D.[23] Metabolic degradation might occur following interaction with the cells in the lymph node, such as lymphocytes and macrophages, but the details remained obscure.

MMC is an antitumor antibiotic that has activity against a number of human neoplasms.[28] However, conventional systemic administration does not provide a sufficient supply of MMC to the tumor with minimal side effects. Local injection of MMC is difficult owing to the consequent severe local tissue damage. However, intratumoral injection of polymeric prodrugs of the antitumor agent is expected to offer a means of overcoming these problems.[29] In the present study, the rat thigh muscle was selected as a model injection site to for precise quantitative analysis. The present pharmacokinetic analysis revealed that a low but consistent level of active MMC was maintained at the injection site following the injection of MMC-D (Fig. 6). Using the simulated values up to 48 h and extrapolation, the area under the time-amount curves of regenerated MMC in the muscle was calculated by the linear trapezoidal method, and the relative bioavailabilities were obtained by comparing them with that of free MMC. The calculated relative bioavailabilities were 27.1% for MMC-D (T-10), 38.7% for MMC-D (T-70), and 46.7% for MMC-D (T-500), and these values are much higher than those obtained by *i.v.* injection.[16]

The cytotoxicity of MMC was reported to be in proportion to the exposure dose (product of the concentration multiplied by the contact time).[30] Furthermore, MMC was found to be selectively toxic to hypoxic tumor cells at low concentrations.[31] The utility of local intratumoral injection of MMC-D is strongly supported by these findings. MMC-D (T-500) seemed to be the most effective agent as a depot which supplies potent MMC gradually at the injection site. In accordance with this consideration, prolonged residence of MMC-D (T-500) in a tumor mass and a corresponding growth-inhibitory effect were observed in rats bearing Walker carcinosarcoma 256.[32]

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