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Development of a novel polymeric prodrug of mitomycin C, mitomycin C–dextran conjugate with anionic charge. II. Disposition and pharmacokinetics following intravenous and intramuscular administration

Yoshinobu Takakura, Ryo Atsumi, Mitsuru Hashida and Hitoshi Sezaki

Department of Basic Pharmaceutics, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto (Japan)

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Summary

Disposition and pharmacokinetics of a novel polymeric prodrug of mitomycin C (MMC), mitomycin C–dextran conjugate with anionic charge (MMC-Dan.), following intravenous (i.v.) and intramuscular (i.m.) injection were studied in rats. Three types of MMC-Dan., conjugates with dextran with molecular weights of 10,000, 70,000, and 500,000 were tested. After i.v. injection, MMC-Dan. showed a exceedingly slow plasma clearance of MMC in the conjugated form, and the disappearance rate was decreased as the molecular weight of the conjugate increased. Urinary excretion was also affected by the molecular weight; i.e. the urinary recovery percent of conjugated MMC was decreased with the increase of the molecular weight of the conjugate. These results suggested that MMC-Dan. was retained in the body for a considerably long period. After i.m. injection, MMC-Dan. disappeared from the injection site with biphasic patterns. In an initial phase, conjugates themselves disappeared with rates depending on their molecular sizes, but thereafter they disappeared at almost the same rate. The release of MMC from subsequent absorption was suggested in the injection site. MMC-Dan. was accumulated in the regional lymph node and transferred to thoracic lymph as their sizes increased and decreased, respectively. The relationship between physicochemical properties, such as molecular weight and electric charge, and the pharmacokinetic behaviour of polymeric prodrug in systemic and local administration was discussed, in comparison with mitomycin C–dextran conjugate with cationic charge (MMC-Dcat.).

Introduction

Recently, various macromolecular drug carrier systems, involving tumor-specific and non-specific carriers, have been widely developed in an attempt to improve the pharmacokinetic behavior of antitumor drugs (Sezaki and Hashida, 1984; Poznansky and Cleland, 1980). The rational for this approach lies in the altered pharmacokinetics of conjugated drug that is predominantly dictated by the nature of macromolecule and consequently differs from the disposition of the parent drug given by the same administration route. Basically, the in vivo disposition of macromolecule–drug

Correspondence: H. Sezaki, Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto 606, Japan.

conjugates, even tumor-specific ones, is controlled by the passive mechanism, relying on the physicochemical interactions with the body. Therefore, physicochemical property of conjugates is one of the most important factors which decide the in vivo fate of the conjugate and consequently the pharmacological efficacy. In spite of numerous reports on the antitumor effect of conjugates, however, little is known about their pharmacokinetics especially in relation to its physicochemical properties.

In our series of investigations, we have developed a polymeric prodrug of mitomycin C, mitomycin C–dextran conjugate with cationic charge (MMC-Dcat.) (Kojima et al., 1980), and examined its disposition and pharmacokinetics after intramuscular (i.m.) (Takakura et al., 1984, 1986) and intravenous (i.v.) (Hashida et al., 1984; Takakura et al., 1985) administrations. In local injection, MMC-Dcat. showed sustained retention at the injection site and enhanced lymphatic delivery. Remarkable reduction in tumor size was obtained in a clinical trial involving intratumoral administration (Honda et al., 1985). However, intravenous treatment of MMC-Dcat. exhibited lower activity as compared to intraperitoneal treatment or topical injection (Hashida et al., 1981; Matsumoto et al., 1985), because of rapid removal from the circulation and consequent low bioavailability of MMC (Hashida et al., 1984). The polycationic nature of MMC-Dcat. seemed to play an important role in these phenomena.

In our recent paper (Takakura et al., 1987), a polymeric prodrug of MMC, MMC–dextran conjugate with anionic charge (MMC-Dan.) was developed, and MMC-Dan. demonstrated to exhibit an excellent in vivo antitumor activity. In the present study, disposition of MMC-Dan. after i.v. and i.m. injection was studied in order to clarify the effect of physicochemical properties of conjugates such as electric charge and molecular weight.

Materials and Methods

Chemicals

MMC was kindly supplied by Kyowa Hakko Kogyo Co. (Tokyo, Japan). Dextrans with various molecular weights were purchased from Pharmacia (Uppsala, Sweden), and had average molecular weights of about 10,000 (T-10), 70,000 (T-70), and 500,000 (T-500). Radiolabeled [^{14}C(U)]γ-aminobutyric acid was obtained from New England Nuclear (Boston, MA), with a specific activity of 2 mCi/mg. All other chemicals were reagent grade products obtained commercially.

Synthesis of MMC-Dan.

Three types of MMC-Dan. were synthesized as reported previously (Takakura et al., 1987). All conjugates were estimated to contain MMC to almost equal extents of about 8% (w/w). Radiolabeled MMC-Dan. was synthesized by coupling [^{14}C]γ-aminobutyric acid (10 μCi) together with MMC at a molar ratio of 1 : 2000. The radioactivity of synthesized MMC-Dan. was determined in a liquid scintillation system. About 4% of added [^{14}C]γ-aminobutyric acid was conjugated with spacer-introduced dextran (20 mg) and the purified radiolabeled MMC-Dan. has a specific activity of 0.02 μCi/mg. The doses reported for MMC-Dan. refer to the quantity of MMC contained in the conjugate. Test compounds were prepared in saline solution at a concentration of 2 mg equivalent MMC/ml for injection.

Procedure of animal experiment with intravenous injection

Male Wistar albino rats (200–250 g), obtained from Shizuoka Agricultural Co-operative Association for Laboratory Animals (Shizuoka), were used in all animal experiments. For determining plasma concentration, the animals were anesthetized with ether, injected with the drug solution into the femoral vein, and were housed in standard animal cages. At various time periods, blood samples were withdrawn from the aorta under ether anesthesia. Blood was centrifuged at 3000 rpm for 5 min and the plasma samples were assayed. For determining urinary excretion, rats having i.v. administration of the drug were housed in metabolic cages. Urine samples were collected at adequate intervals in cooled test-tubes and stored in a freezer until assay.

Procedure of animal experiment with intramuscular injection

The rats were anesthetized with ether and injected with 100 μl aliquots of saline solution of the test compounds into the center of the left thigh muscle using a microsyringe. At various time periods after the injection, rats were anesthetized with ether, and the thigh muscle and regional lymph node (left iliac node) (Tilney, 1971) 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. For thoracic lymph collection, rats were anesthetized with sodium pentobarbital, and the thoracic duct was cannulated according to the method of Bollman et al. (1948). 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 restaining 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 concentration of MMC in plasma, urine, and lymph fluid was determined by microbiological assay. The thigh muscle and the lymph node were homogenized and subjected to bioassay. Assay was performed by a disc-plate method using *E. coli B* as a test organism. The concentration of free and conjugated form of MMC were determined as reported previously (Hashida et al., 1984). In brief, the sample containing free and conjugated MMC was divided into two groups and each sample was diluted with saline and pH 8.0 phosphate buffer. The former was directly subjected to assay in which free MMC was detected predominantly. The latter was heated in order to hydrolyze conjugated MMC to free MMC and the total MMC was determined. The concentration of free and conjugated MMC was estimated by calculation based on the calibration curves.

The amount of carrier dextran in urine was determined by the same procedure as described in a previous report (Takakura et al., 1985). The dextran in urine samples was measured by anthrone method after deproteinization with trichloroacetic acid and precipitation by ethyl alcohol (Roe, 1954). The procedure for the determination of ^{14}C-radioactivity was a modification of the method of Mahin and Loftberg (1966). 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. The lymph nodes and thoracic lymph were assayed without solubilization with NaOH. Then, 0.2 ml of muscle sample solution, 0.2 ml of thoracic lymph fluid, and weighed lymph nodes were 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 medium (Univer-gel; Nakarai Chemicals, Kyoto) was added and the radioactivity was determined in a liquid scintillation system (Beckman, LS-232).

Pharmacokinetic analysis

The data on disappearance from the injection site of MMC-Dan. (T-70) were analyzed using a one-compartment model with a Langmuir-type adsorption step as reported previously (Takakura et al., 1987). The following differential equations 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.

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

$$dX_2/dt = -k_a \cdot X_{2,f} - k_r \cdot X_2$$

$$= -k_a \cdot X_{1,f} \cdot X_2/X_1 - k_r \cdot X_2 \tag{2}$$

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

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:

$$X_1 = X_{1,f} + X_{1,b}$$
$$= X_{1,f} + X_\infty \cdot K \cdot X_{1,f}/(1 + K \cdot X_{1,f}) \quad (4)$$

where $X_{1,b}$, K, and X_∞ are the amount of bound carrier dextran, the binding constant, and the maximum amount of carrier dextran that can be absorbed on the muscle, respectively. The quadratic from Eqn. 4 gives:

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

Substituting Eqn. 5 into Eqns. 1 and 2 gives:

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

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

The muscle clearance data of the carrier dextran shown and those of the conjugated form of MMC were fitted simultaneously to Eqns. 6 and 7, respectively, and the parameters, k_a, X_∞, K, and k_r were determined by using the non-linear least-squares program MULTI(RUNGE) (Yamaoka et al., 1983). The amount of free MMC regenerated in the muscle was calculated based on Eqn. 3 using the elimination constant of free MMC (k_e = 9.20 h^{-1}) determined in the previous absorption study of free MMC (Takakura et al., 1984). Using the calculated values up to 48 h with extrapolation, the area under the time–amount curve of regenerated MMC in the muscle was determined by the linear trapezoidal method.

Results

Plasma concentration following i.v. injection

Fig. 1 shows the plasma concentration of MMC after i.v. injection of free MMC and MMC-Dan. with various molecular weights. The plasma concentration of MMC after injection of free MMC decreased rapidly with a half-life of about 8.6 min. In the case of MMC-Dan., free and conjugated forms of MMC could not be measured separately because the concentration of conjugated MMC was too high to detect the free MMC accurately by bioassay. Therefore, only the total concentration, which predominantly corresponds to the concentration of conjugated MMC, is shown in Fig. 1. Prolonged plasma concentration was observed after i.v. injection of MMC-Dan., and elimination rate from the circulation altered depending on the molecular weight. MMC-Dan. (T-500) exhibited the slowest elimination (half-life = 8 h) and the concentration was 8 μg/ml even at 24 h after administration.

Urinary excretion following i.v. injection

Fig. 2 shows the cumulative amount of free and conjugated MMC excreted in urine after i.v. injection of MMC-Dan. (T-10) (A), MMC-Dan. (T-70)

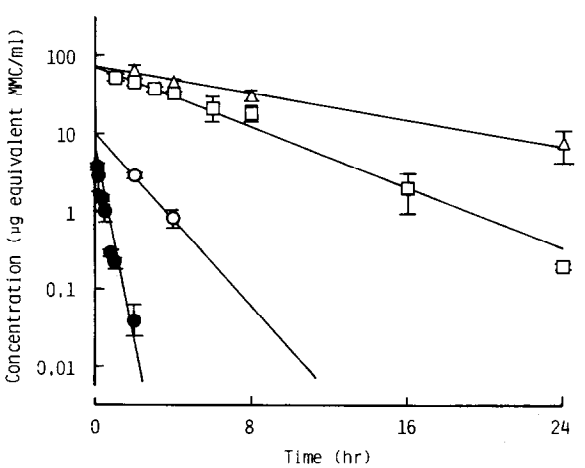

Fig. 1. Plasma concentration of MMC after intravenous injection of MMC and MMC-Dan. with various molecular weights. Results are expressed as the mean ± S.D. of 3 or 4 rats. Key: ●, MMC; ○, MMC-Dan. (T-10); □, MMC-Dan. (T-70); △, MMC-Dan. (T-500).

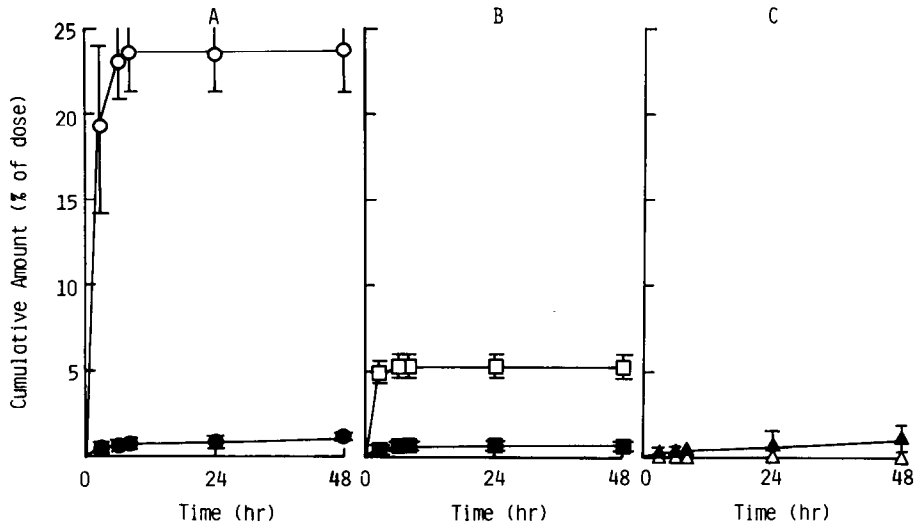

Fig. 2. Cumulative amount of free and conjugated forms of MMC excreted in urine after intravenous injection of MMC-Dan. (T-10) (A), MMC-Dan. (T-70) (B), and MMC-Dan. (T-500) (C). Results are expressed as the mean ± S.E. of 3 or 4 rats. Key: ● ■ ▲, free MMC; ○ □ △, conjugated MMC.

(B), and MMC-Dan. (T-500) (C). The amount of conjugated MMC excreted in urine was decreased as the molecular weight of the conjugate increased. That is, 23.5% and 5.3% of doses were recovered in urine after injection of MMC-Dan.

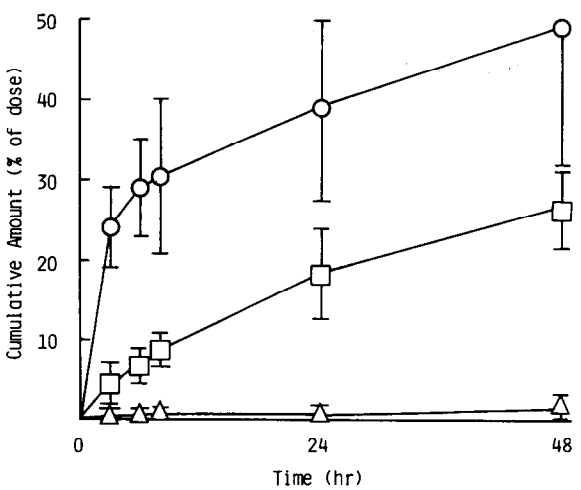

Fig. 3. Cumulative amount of carrier dextran excreted in urine after intravenous injection of MMC-Dan. with various molecular weights. The concentration of dextran was determined by the anthrone method. Results are expressed as the mean ± S.E. of 3 or 4 rats. Key: ○, MMC-Dan. (T-10); □, MMC-Dan. (T-70); △, MMC-Dan. (T-500).

(T-10) and MMC-Dan. (T-70), respectively, whereas little was recovered in the case of MMC-Dan. (T-500). On the other hand, urinary excretion of free MMC gave almost the same pattern regardless of molecular size of MMC-Dan. Fig. 3 shows the cumulative amount of carrier dextran excreted in urine after i.v. injection of MMC-Dan. The effect of molecular weight on the urinary excretion was similar to the results obtained for conjugated MMC by bioassay shown in Fig. 2. In this case, continuous excretion of dextran was observed up to 48 h after administration of MMC-Dan. (T-10) and MMC-Dan. (T-70). Carrier dextran was scarcely recovered in urine after injection of MMC-Dan. (T-500).

Absorption from the injection site following i.m. injection

Fig. 4A shows the disappearance of MMC from the thigh muscle after i.m. injection of MMC-Dan. with various molecular weights. Since free MMC was absorbed very rapidly, the amounts of MMC in these experiments were considered to correspond to the conjugated MMC (Takakura et al., 1984). MMC-Dan. was absorbed biphasically and the disappearance rates of MMC in the initial phase were increased as their molecular weights

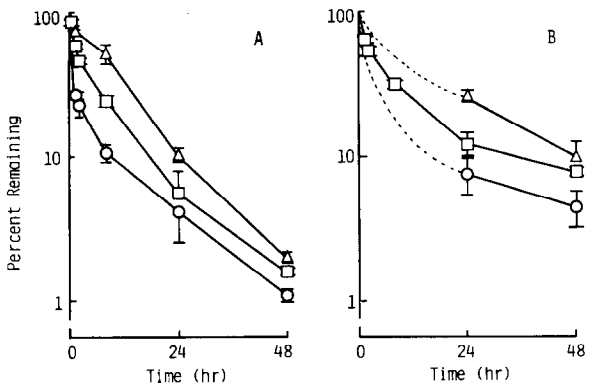

Fig. 4. Disappearance of conjugated MMC (A) or carrier dextran ([^{14}C]MMC-Dan.) (B) from the injection site after i.m. injection of MMC-Dan. or [^{14}C]MMC-Dan. Results are expressed as the mean ± S.E. of 3 of 4 rats. Key: ○, MMC-Dan. (T-10); □, MMC-Dan. (T-70); △, MMC-Dan. (T-500).

decreased. However, they showed almost similar clearance rates in the second half period studied. Fig. 4B shows the disappearance of radioactivity from the injection site after injection of 3 types of [^{14}C]MMC-Dan. The radiolabelling of MMC-Dan. was confirmed to be stable by a stability study, so that the radioactivity was considered to show the behaviour of carrier dextran in MMC-Dan. Dependence of absorption rate on molecular weight

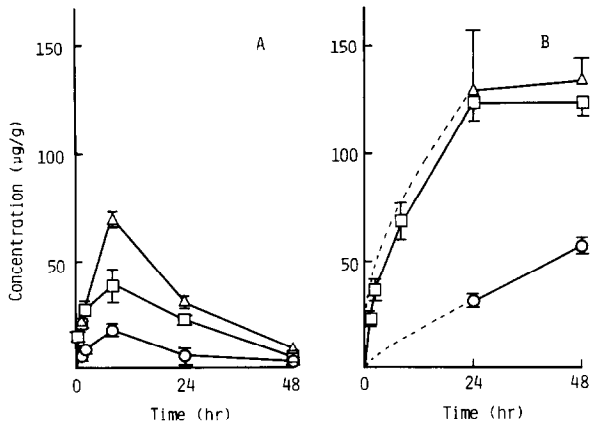

Fig. 5. Iliac lymph node concentration of conjugated MMC (A) or carrier dextran ([^{14}C]MMC-Dan.) after i.m. injection of MMC-Dan. or [^{14}C]MMC-Dan. Results are expressed as the mean ± S.E. of 3 or 4 rats. Key: ○, MMC-Dan. (T-10); □, MMC-Dan. (T-70); △, MMC-Dan. (T-500).

TABLE 1

The concentration ratio of MMC to dextran in the thigh muscle and iliac lymph node after intramuscular injection of MMC-Dan. with various molecular weights

Time (h)	MMC-Dan. (T-10)	MMC-Dan. (T-70)	MMC-Dan. (T-500)
Muscle			
1	–	0.906	–
2	–	0.960	–
8	–	0.828	–
24	0.544	0.512	0.644
48	0.285	0.276	0.211
Lymph node			
1	–	0.918	–
2	–	0.691	–
8	–	0.608	–
24	0.196	0.188	0.237
48	0.063	0.032	0.059

Each MMC/dextran ratio was calculated from the data shown in Figs. 4 and 5.

was also observed, but the elimination of carrier dextran was slower than that of MMC in all cases.

In order to clarify the regeneration characteristics of MMC-Dan. at the injection site and the lymph node after i.m. injection, time courses of concentration ratio of MMC to dextran was calculated from the results shown in Figs. 4 and 5, and the results are summarized in Table 1. The ratios in the thigh muscle were decreased gradually from 1 but they kept the values of more than 0.5 for all MMC-Dan.s even 24 h after injection. These results suggest that MMC conjugated with dextran in its form was stable and only a step of hydrolytic release of MMC proceeded in the muscle.

Regional lymph node uptake

Fig. 5A illustrates the iliac lymph node concentration of MMC following the i.m. injection of MMC-Dan. Free MMC was not detected in the lymph node in all experiments. Remarkable uptake of conjugated MMC was observed in the lymph node after the injection of all types of MMC-Dan. All MMC-Dan.s gave the same pattern, that is, the peak was obtained 8 h after injection. The peak value was increased with the

TABLE 2

Plasma concentration of MMC in conjugated and free form after intramuscular injection of MMC-Dan. with various molecular weights

Time (hr)	MMC-Dan. (T-10)	MMC-Dan. (T-70)	MMC-Dan. (T-500)
Conjugated MMC (μg/ml)			
1	0.163±0.026(3)	N.D. [a]	N.D.
2	0.063±0.030(3)	N.D.	N.D.
8	N.D.	N.D.	N.D.
24	N.D.	N.D.	N.D.
48	N.D.	N.D.	N.D.
Free MMC (μg/ml)			
1	0.036±0.002(3)	Trace [b]	Trace
2	0.019±0.006(3)	Trace	Trace
8	Trace	Trace	Trace
24	Trace	Trace	Trace
48	Trace	Trace	Trace

[a] Not detected.

[b] Trace amount was detected.

Results are expressed as the mean±S.D. and values in parentheses are number of rats.

increase of molecular weight. The lymph node uptake of carrier dextran after i.m. injection of [^{14}C]MMC-Dan. is shown in Fig. 5B. Carrier dextrans were obviously accumulated in the lymph node depending on their molecular sizes. Rapid decrease in the concentration ratio of conjugated MMC to [^{14}C]MMC-Dan. was observed in the lymph nodes shown in Table 1, suggesting that MMC in MMC-Dan. underwent a rapid inactivation in the lymph node.

Transfer to the thoracic lymph following i.m. injection

Fig. 6 shows the cumulative amount of free and conjugated forms of MMC transferred to the thoracic lymph following i.m. injection of MMC-Dan. (T-10) (A), MMC-Dan. (T-70) (B), and MMC-Dan. (T-500) (C). Average lymph flow during the course of the experiment is also shown. The recovery of the conjugated form of MMC decreased with increase of molecular weight, and 3.1, 1.6, and 0.3% of the doses were recovered within 48 h after injection of MMC-Dan. (T-10), MMC-Dan. (T-70), and MMC-Dan. (T-500), respectively. The cumulative amount of free MMC was about one-third of that of conjugated MMC in the case of MMC-Dan. (T-10) and MMC-Dan. (T-70). On the contrary, free MMC was predominantly transferred into thoracic lymph fluid after the injection of MMC-Dan. (T-500). Results obtained for carrier dextran after i.m. injection of

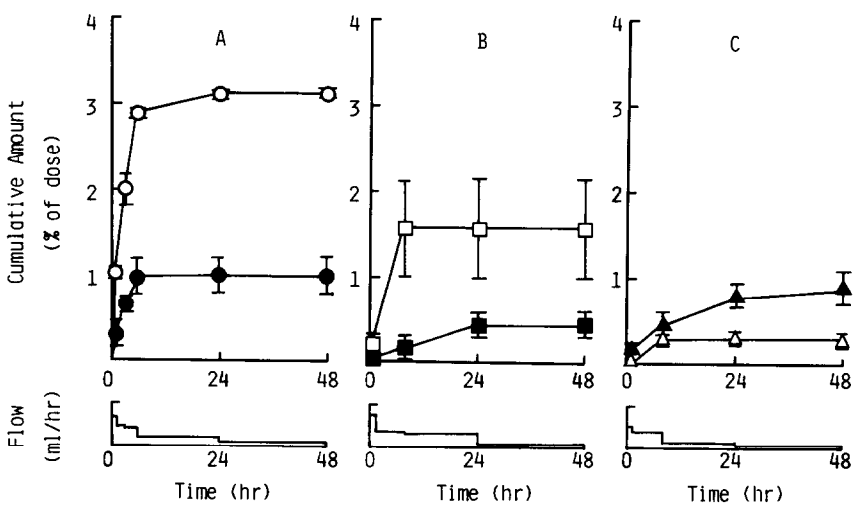

Fig. 6. Cumulative amount of free (closed symbols) and conjugated MMC (open symbols) transferred to the thoracic lymph after i.m. injection of MMC-Dan. (T-10) (A), MMC-Dan. (T-70) (B), and MMC-Dan. (T-500) (C) and average lymph flow. Results are expressed as the mean ± S.E. of 3 rats.

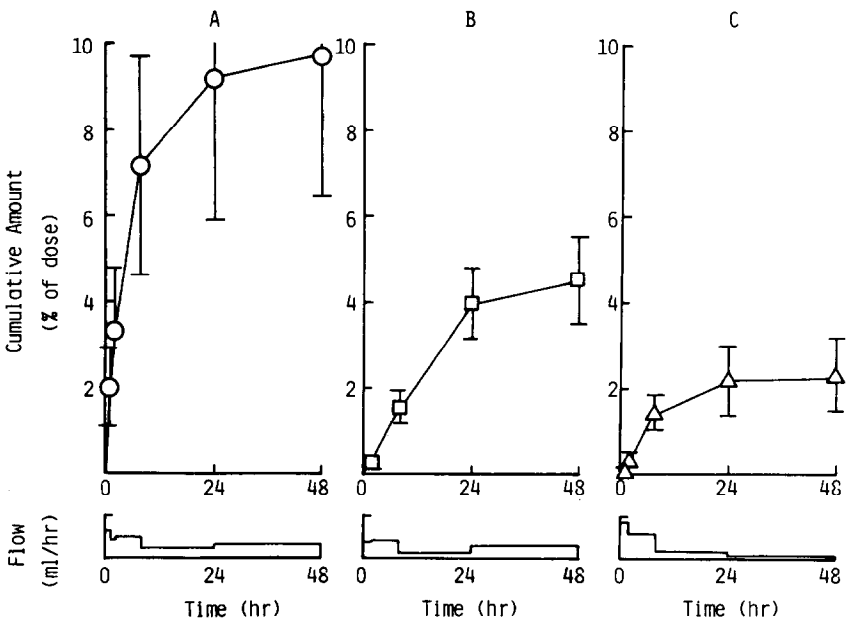

Fig. 7. Cumulative amount of carrier dextran transferred to thoracic lymph after i.m. injection of [^{14}C]MMC-Dan. (T-10) (A), [^{14}C]MMC-Dan. (T-70) (B), and [^{14}C]MMC-Dan. (T-500) (C) and average lymph flow. Results are expressed as the mean ± S.E. of 3 rats.

three types of [^{14}C]MMC-Dan. are shown in Fig. 7. Molecular weight gave the same effect on the transfer of carrier dextran as on that of conjugated MMC. However, a considerable amount was continuously recovered in thoracic lymph even during the second 24 h in the case of carrier dextran.

TABLE 3

Computer-estimated pharmacokinetic parameters for disappearance of MMC-Dan. (T-70) and MMC-Dcat. (T-70) from the injection site after intramuscular injection

Parameters	MMC-Dan. (T-70)	MMC-Dcat. (T-70)
k_a (h^{-1}) [a]	0.741	0.702
X_∞ (% of dose) [b]	40.1	42.7
K ((% of dose)$^{-1}$) [c]	0.763	22.0
k_r (h^{-1}) [d]	0.0345	0.0247
AUC (% of dose h) [e]	2.30	4.21

[a] Absorption rate constant.
[b] Maximum amount of adsorption.
[c] Binding constant.
[d] Regeneration rate constant from MMC-D to MMC.
[e] Area under the curve of regenerated MMC.

Plasma concentration following i.m. injection

Table 2 shows the plasma concentration of MMC after i.m. injection of MMC-Dan. MMC was not detected as the conjugated form in plasma following the administration of MMC-Dan. except for MMC-Dan. (T-10). Only a trace level of free MMC was recovered up to 48 h after administration of all types of MMC-Dan.

Pharamacokinetical analysis of absorption behaviour of MMC-Dan.

In order to clarify the absorption mechanism, pharmacokinetic analysis was carried out for MMC-Dan. (T-70) using a compartment model including a Langmuir-type adsorption in the muscle (Takakura et al., 1986). The computer-generated parameters for MMC-Dan.(T-70) are summarized in Table 3 together with those for MMC-Dcat. (T-70) obtained in our previous study (Takakura et al., 1986). Absorption rate constants

(k_a) were almost the same, suggesting that the absorption rate is mainly governed by the molecular weight. On the contrary, binding constant (K) of MMC-Dan. was smaller than those of MMC-Dcat., implying that anionic charge may inhibit the absorption of MMC-Dan. to the muscle. AUC of regenerated MMC at the injection site was calculated to be about a half of that of MMC-Dcat. (T-70) in the case of MMC-Dan. (T-70).

Discussion

In the present study, disposition of MMC-Dan. following i.v. administration was studied in order to clarify the superior in vivo antitumor activity (Takakura et al., 1987). Furthermore, the fate of MMC-Dan. after i.m. injection was investigated for assessing the utility in local administration.

In the i.v. administration experiment, MMC-Dan. showed almost monoexponential plasma clearance and long circulation life time as shown in Fig. 1. The molecular weights remarkably affected the clearance, and MMC-Dan. (T-500) was found to have the longest circulating life. Urinary excretion of conjugated MMC (Fig. 2) and carrier dextran (Fig. 3) also depended on the molecular weight, that is, the excreted amount was restricted as the molecular weight increased. This phenomenon was in good agreement with the plasma clearance data. Successive release of MMC from MMC-Dan. in the body was also confirmed from the urine data.

Our previous study (Hashida et al., 1984) revealed that MMC-Dcat. showed biexponential and relatively rapid clearance after i.v. injection, and the clearance rate was increased as the molecular weight increased. On the contrary, a reverse effect of molecular weight on the clearance rate was observed for MMC-Dan. (Fig. 1). These findings suggest that the molecular weight has an opposite influence on the in vivo behaviour of polymeric prodrugs on their electric charges. Slow plasma clearance of MMC-Dan. implies that tissue distribution of MMC-Dan. might be slow as compared with MMC-Dcat., which was confirmed to be rapidly accumulated to the reticuloendothelial systems such as liver and spleen (Hashida et al., 1984). Our preliminary study on in vivo distribution of [^{14}C]MMC-Dan. supported this speculation (unpublished data). The disposition characteristics of MMC-Dan. in systemic administration corresponded well to an excellent in vivo antitumor effect of MMC-Dan. shown in the previous report (Takakura et al., 1986a). Larger MMC-Dan. was considered to act as a potent reservoir of MMC with the longest biological life time and resulted in the superior activity in the i.v.–i.v. system.

In the local administration study, MMC-Dan. exhibited relatively slow disappearance from the injection site (Fig. 4) and remarkable lymphatic transfer (Figs. 5–7) while free MMC was absorbed with a half-life of 5 min under the same conditions (Takakura et al., 1984). The results shown in Table 2 suggested that the main absorption route of MMC-Dan. except MMC-Dan. (T-10) was not the capillaries but the lymphatic vessels. Molecular weight of the conjugate seemed to play an important role in the absorption process of MMC-Dan. following i.m. injection. MMC-Dan. (T-500) exhibited the longest residence in the muscle and the largest lymph node uptake, while its thoracic lymph transfer was restricted. Therefore, MMC-Dan. (T-500) was considered to be absorbed through the lymphatic capillaries and accumulated in the lymph node. On the other hand, MMC-Dan. (T-10) was suggested to be rapidly absorbed by both lymphatic and vascular routes, and transferred into thoracic lymph fluid passing through the lymph nodes.

Comparing the absorption behaviour after the local injection, MMC-Dan. exhibited faster disappearance from the injection site and smaller accumulation in the regional lymph node than MMC-Dcat. Pharmacokinetic analysis of the disappearance patterns of MMC-Dan. and MMC-Dcat. revealed that the binding capacity of conjugates based on the electrostatic force was a predominant factor for the residence of the conjugates in the muscle (Table 3). This fact may also explain the relatively smaller accumulation of MMC-Dan. in the lymph node (Takakura et al., 1984). Consequently, the anionic charge in MMC-Dan. is concluded to lead to a decrease in tissue interaction as well as the interaction with the tumor cells

(Takakura et al., 1987) and bioavailability of active MMC, and thus results in lowering of therapeutic activity in a local treatment.

The present study revealed that MMC-Dan. acts as a reservoir of MMC which behaves characteristically as a macromolecule while supplying active MMC in the body. The disposition characteristics of MMC-Dan. seems to be advantageous for the systemic treatment, since it is retained in the blood pool and supplies active MMC for a long period. Barlogie and Drewinko (1980) showed that the cytotoxicity of MMC is related to the given exposure dose (product of the concentration multiplied by the contact time). This finding suggests that sustained release of MMC from MMC-Dan. in the body cavity played an important role in the expression of the superior antitumor of MMC-Dan. to free MMC, as shown in our previous paper (Takakura et al., 1987). On the other hand, MMC-Dan. showed less activity than MMC-Dcat. in the local treatment, because its accumulation ability in the injection site or the regional lymph node is inferior to that of MMC-Dcat. The present investigation has thus shown that physicochemical characteristics play an important role in determining the in vivo fate and therapeutic potential of macromolecular prodrugs.

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