Disposition Characteristics of Mitomycin C-Dextran Conjugate in Normal and Tumor-bearing Muscles of Rabbits

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ABSTRACT

Disposition characteristics of the macromolecular prodrug of mitomycin C (MMC), mitomycin C-dextran conjugate (MMC-D), in normal and tumor (VX2 carcinoma)-bearing thigh muscles were studied using the in situ vascular perfusion technique. Three types of cationic MMC-D (MMC-Dc) and two types of anionic MMC-D (MMC-Da) with different carrier molecular weights were used. After bolus arterial injection in normal muscles, 83-96% of injected MMC-D was recovered in the venous outflow regardless of the carrier size or charge, whereas less than 60% of MMC was recovered in the same system. By applying statistical moment analysis to the outflow pattern of these drugs, pharmacokinetic parameters representing their disposition characteristics were obtained. Smaller intrinsic clearance (CLin) and distribution volume (Vd) were noted for MMC-D than for MMC, indicating low extravascular diffusion of MMC-D.

In the tumor-bearing muscle, blood contamination from other parts of the body increased and a shortage of flow recovery due to the neovascularization of the tumors occurred. The disposition parameters of MMC-Dat with a molecular weight of 500,000 (T-500) indicated some tissue distribution and sequestration in the tumor preparation.

After constant infusion of [14C]MMC-D (T-500) for 4 h, tissue radioactivity concentrations were determined in various tissues. A higher radioactivity was observed in the viable region of the tumor and the lymph node compared with the normal muscle tissue and the necrotic region of the tumors. 131I-Labeled human serum albumin also gave similar results.

In conclusion, higher tumor localization of antitumor agents may be made possible by the application of macromolecular prodrugs.

INTRODUCTION

In cancer chemotherapy, it is important to control the pharmacokinetic behavior of cytotoxic drugs for effective treatment. Various approaches have been used to concentrate the cytotoxicity of the drug to tumor cells such as by modifying their biopharmaceutical properties.

Previously, we developed macromolecular prodrugs of mitomycin C, mitomycin C-dextran conjugates having cationic or anionic charges by coupling MMC1 to dextran employing ε-aminoacaproic acid or 6-bromohexanoic acid as a spacer, respectively (1, 2). MMC-D has higher activity than MMC against various murine tumors (3) and the nature of the carrier dextran was found to affect its efficiency (4). After systemic administration, MMC-D was retained in the blood circulation for a considerably long period and accumulated in the liver depending on its physicochemical properties (1, 2). In local administration, MMC-D remained at the injection site for a long period and lymphatic delivery was enhanced (5, 6). Although these studies have demonstrated the unique pharmacokinetic characteristics of MMC-D in vivo, little is known about the disposition behavior of MMC-D in a single organ especially at the vascular level.

In the present study, we selected the muscle of rabbits as a model organ and examined the disposition characteristics of MMC-D in its vascular bed. Using the in situ single-pass perfusion technique, drugs were introduced as a pulse function from the arterial side and venous outflow patterns were analyzed by statistical moment analysis to derive the disposition parameters (7).

A number of investigators have reported that macromolecules such as albumin or dextran accumulate in tumors because of leakiness of tumor neovasculature and lack of lymphatic systems (8-11). Therefore, macromolecular prodrugs of antitumor drugs may be useful in delivering the drug to the tumor site. To assess the feasibility of MMC-D as a drug delivery system for tumors (12), VX2 carcinoma was implanted in the rabbit thigh muscle and tissue accumulation of MMC-D was determined in the same manner.

MATERIALS AND METHODS

Chemicals. Mitomycin C was kindly supplied by Kyowa Hakko Kogyo Co., Tokyo, Japan. Dextran of various molecular weights were purchased from Pharmacia Fine Chemicals Co., Uppsala, Sweden, and had average molecular weights of about 10,000 (T-10), 70,000 (T-70), and 500,000 (T-500). γ-Aminobutyric acid with a specific radioactivity of 2 mCi/mg was purchased from New England Nuclear, Boston, MA, and 131I-labeled HSA with a specific radioactivity of 1 mCi/ml was purchased from Daiichi Radioisotope, Tokyo. All other chemicals were reagent grade products obtained commercially.

Preparation of MMC-D. MMC-Dat was synthesized as reported previously (1). In brief, dextran was activated by cyanogen bromide at pH 10.7 and ε-aminoacaproic acid was coupled covalently to a glucose chain as a spacer. The product was washed repeatedly by ultrafiltration and MMC was conjugated by a carbodiimide catalyzed reaction. The product was washed, and MMC was coupled in the same manner as MMC-Dat. In the present experiment, three types of MMC-Dat (T-10, T-70, and T-500) and two types of MMC-Dab (T-10 and T-70) were used. Radiolabeled MMC-Dab (T-500) was synthesized using γ-amino[U-14C]butyric acid as described previously (13). Fig. 1 and Table 1 show the structure and physicochemical properties of MMC-Dat and MMC-Dab.

Animals and Tumors. Male domestic rabbits each weighing 1.9-2.1 kg fed a commercial diet were used. VX2 carcinoma grown in the gluteal muscle of the rabbit was excised, and necrotic areas and connective tissue were removed. The tumor was minced in Hanks' solution with scissors and was made into a cell suspension at a concentration of 20% (v/v). The suspension (0.3 ml) was inoculated into the right posterior thigh muscle 2-3 centimeters above the poples. The tumor was used for the perfusion experiment when it had grown to 4-6 cm in diameter, about 14-21 days after the inoculation.

Perfusion Experiment. The rabbit hind leg was perfused as reported previously (7). Fig. 2 shows a diagram of the perfusion system. Briefly, one of the rabbit's hind legs was isolated by ligation all vessels connected to the leg. The femoral artery and vein were cannulated with vinyl tubes and perfused at a rate of 1.66 ml/min. There was good recovery of...
perfusion (85.6 ± 3.9%), and the contamination of blood from other parts of the body was only 5.0 ± 1.1% in the normal rabbit. The drug was introduced from the arterial side using a six position rotary valve injector (type 50 Teflon rotary valves, Rhodyne, CA) as a pulse function. The tissue preparation was perfused with Tyrode's solution (14) (a mixture of 137 mM NaCl, 2.68 mM KCl, 1.80 mM CaCl₂, 11.9 mM NaHCO₃, 0.362 mM NaH₂PO₄, 0.492 mM MgCl₂, and 5.55 mM D-glucose) containing bovine serum albumin (Fraction V; Armour Pharmaceutical Co., Eastbourne, Sussex, UK) at a concentration of 4.7% (w/v). The perfusate was gassed with 95% O₂-5% CO₂ and maintained at 37°C. At the end of the experiment, the leg was perfused with a 0.9% NaCl solution of Evans blue and the stained part of the muscle was excised after the animal was killed. The mean weight of the perfused area was 29.8 ± 6.7 g.

Table 1  Physicochemical characteristics of MMC-D

<table>
<thead>
<tr>
<th>MMC-D&lt;sub&gt;car&lt;/sub&gt; (T-10)</th>
<th>MMC-D&lt;sub&gt;car&lt;/sub&gt; (T-70)</th>
<th>MMC-D&lt;sub&gt;car&lt;/sub&gt; (T-500)</th>
<th>MMC-D&lt;sub&gt;car&lt;/sub&gt; (T-10)</th>
<th>MMC-D&lt;sub&gt;car&lt;/sub&gt; (T-70)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M&lt;sub&gt;c&lt;/sub&gt; (carrier)</td>
<td>Mitomycin C content</td>
<td>Release&lt;sup&gt;a&lt;/sup&gt; T&lt;sub&gt;n&lt;/sub&gt; (h)</td>
<td>% Adsorption at pH 7.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>CM-Sephadex</td>
</tr>
<tr>
<td>9,900</td>
<td>10.80</td>
<td>24.4</td>
<td>51.3</td>
<td>0</td>
</tr>
<tr>
<td>64,400</td>
<td>8.46</td>
<td>23.6</td>
<td>61.7</td>
<td>0</td>
</tr>
<tr>
<td>487,000</td>
<td>10.10</td>
<td>23.8</td>
<td>50.0</td>
<td>0</td>
</tr>
<tr>
<td>9,900</td>
<td>9.43</td>
<td>35.0</td>
<td>0</td>
<td>29.2</td>
</tr>
<tr>
<td>64,400</td>
<td>8.15</td>
<td>35.4</td>
<td>0</td>
<td>32.0</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determined by the Visking tube dialysis method in pH 7.4 phosphate buffer at 37°C (2, 4).
<sup>b</sup> Estimated by the batch method at pH 7.2 (2, 19).

Pharmacokinetic Analysis of Outflow Pattern. Moment analysis was used for analyzing the outflow pattern, the detailed theoretical description of this analysis was given previously (7). For understanding the parameters derived from this analysis, several points should be considered: (a) moment analysis regards the perfused organ as a black box in which the distribution and elimination of drugs occur assuming a linear condition for the concentration of the drug. (b) The disposition function of this system is reflected in output response following a unit pulse input; i.e., in the concentration-time course in outflow. (c) The first three (zero to second) statistical moment parameters (moments) for output response are defined as follows,

\[ \text{auc} = \int C \, dt \]  
\[ T = \frac{\int C \, dt}{\text{auc}} \]  
\[ \sigma^2 = \int (t - T)^2 C \, dt/\text{auc} \]

where \( t \) is time and \( C \) is the concentration of a substance normalized by injection dose with dimension of “% of dose/ml” and \( \text{auc} \), \( T \), and \( \sigma^2 \) are the area under the concentration-time curve, the mean transit time, and the variance of transit time, respectively. (d) The moments are calculated by numeral integration using a linear trapezoidal formula from the outflow concentration-time curve. (e) To obtain background information about the tissue preparation, we used a VRS consisting of albumin labeled with Evans blue which does not distribute to the extravascular space and is not eliminated. (f) Disposition parameters are calculated from the moments. Table 2 shows the derivation of the parameters. They can be divided into two groups, that is, parameters representing distribution (\( V \), \( k \)) and parameters representing elimination (\( F \), \( CL_{\text{tot}} \), \( t_a \)).

Table 2  Derivation of the disposition parameters from moments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distribution</td>
<td>( V = Q / T_a )</td>
</tr>
<tr>
<td>Distribution volume</td>
<td>( k = (1 / T_a - 1) )</td>
</tr>
<tr>
<td>Tissue distribution ratio</td>
<td>( F = \frac{\text{auc of the test drug}}{\text{auc of VRS}} )</td>
</tr>
<tr>
<td>Elimination</td>
<td>( CL_{\text{tot}} = \frac{V}{T_a} )</td>
</tr>
<tr>
<td>Recovery</td>
<td>( t_m = \frac{T_a}{1 - F} )</td>
</tr>
<tr>
<td>Intrinsic clearance</td>
<td>( (1 / T_a) )</td>
</tr>
<tr>
<td>Mean elimination time</td>
<td>( t_m = \frac{T_a}{1 - F} )</td>
</tr>
</tbody>
</table>

\(<sup>4</sup> \( Q \), flow rate; \( k \), ratio of mass of drug in tissue to that in the blood and independent of perfusion area; \( CL_{\text{tot}} \), maximal ability of elimination of the local perfusion system, independent of the perfusate flow rate; \( t_m \), mean time necessary for drug to be eliminated from the perfusate compartment; \( T_a \), mean transit time of VRS.\n
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Local Disposition of Mitomycin C-Dextran

Constant Infusion of the Tumor-bearing Preparation. [14C]MMC-Dc,m (T-500) or 131I-labeled HSA was infused in the same system as in the bolus perfusion experiment except for the injector. Both substances were dissolved in the perfusate and infused for 4 h. At the end of the infusion, the drug-free perfusate was infused for 15 min and the remaining drug was washed out within the intravascular space. The perfusate flowing out in the last 1 min of wash out was collected and used for the assay. After washing, the animal was killed and the muscle, the viable and necrotic regions of the tumor, and the popliteal lymph node were excised. The necrosis fluid in the cavity of the tumor mass was also collected. Each tissue sample was minced into small pieces (about 0.2 g). The procedure employed for the determination of 14C radioactivity was a modification of the method of Mahin and Loftberg (15). The sample was put into a counting vial and 0.2 ml of perchloric acid (60%) and 0.2 ml of hydrogen peroxide (35%) were added. The resulting mixture was heated at 70°C for 90 min with agitation. The mixture was cooled to room temperature, and 5 ml of scintillation medium (Univer-Gel, Nakarai Chemicals) was added to it. The radioactivity was determined in a liquid scintillation system. 131I Radioactivity was directly determined by a NaI well-type scintillator.

RESULTS

Outflow Patterns in the Normal Muscle. Fig. 3 shows the typical concentration-time curves of MMC and MMC-Dc,m (T-70) in the first 3 min, together with that of the VRS, Evans blue-labeled albumin, in the same tissue preparation. The concentration of MMC in the outflow was lower than that of VRS, whereas that of MMC-Dc,m (T-70) was almost the same. Outflow patterns of other MMC-Ds with different charges and carrier sizes are not shown in the figures but were similar to those of MMC-Dc,m (T-70) except for MMC-Dc,m (T-500). The peak outflow concentration of MMC-Dc,m (T-500) was delayed and decreased, compared to that of VRS (Fig. 4).

Moments and Disposition Parameters for Results Obtained in the Normal Muscle. Table 3 summarizes the first three moments and disposition parameters for MMC, MMC-D, and VRS. Almost all of the injected MMC-D was found in the outflow regardless of charge or carrier size, whereas less than 60% of MMC was recovered. Smaller CLm and larger Tm values also indicated smaller elimination of MMC-D. MMC-D showed a smaller distribution volume (V) than MMC also at all charges and carrier sizes examined. The tissue distribution ratio (k) showed this trend more clearly; i.e., k values of MMC-Ds were one-third to one-fourth those of MMC, which indicates the relatively low diffusive character of MMC-D.

Moments and Disposition Parameters for the Results Obtained in the Tumor-bearing Muscle. Though the tumor-bearing preparation was perfused in the same manner as the normal preparation outflow, recovery was decreased from the normal value (85.6 to 70.3%) and more blood came from other parts of the body (5.0 to 12.8%). This incompleteness in tissue isolation may be attributable to the neovascularization of the tumor, which is usually noted in tumor-bearing preparations. No difference in moments of VRS was observed between the tumor-bearing preparation and the normal preparation. In the case of MMC-Dc,m (T-500), however, drug recovery (F) was lower and the distribution volume (V) was higher in the tumor-bearing tissue than those in the normal tissue (Table 4).

Constant Infusion of Macromolecules in the Tumor-bearing Preparation. To assess the slower distribution to various tissues, [14C]MMC-Dc,m (T-500) was dissolved in the perfusate and constantly infused. After a 4-h single-pass infusion, higher radioactivity was observed in the viable region of the tumor than in the necrotic region, the necrosis fluid of the tumor, or the normal muscle tissue. Little radioactivity was detected in the outflow fluid after a 15-min wash out with drug-free perfusate (Fig. 5). High radioactivity was also noted in the popliteal lymph node. In another experiment, 131I-labeled HSA was infused in the same manner as [14C]MMC-Dc,m (T-500). The tissue distribution of 131I-labeled HSA was analogous to that of [14C]MMC-Dc,m (T-500) (Fig. 6).

DISCUSSION

The purpose of this study was to elucidate the pharmacokinetic behavior of MMC-Ds in the muscle capillary system in relation to their physicochemical properties, and to clarify the difference in vascular permeability to macromolecules between the normal and tumor-bearing muscles.

We selected the muscle as a model organ because it comprises more than 50% of the total body weight (16) and seems to play an important role in drug disposition. Another reason is that this tissue has no particular function in metabolism or excretion; thus, it can be used to examine the effect of disease states such as malignancy or inflammation.

The muscle has continuous endothelial capillaries (17) that have little permeability to macromolecules (18). In the present study, MMC-Ds were found to have a smaller distribution volume (V) and intrinsic clearance (CLm) than MMC in the normal muscle (Table 3). These results suggest that MMC-D
LOCAL DISPOSITION OF MITOMYCIN C-DEXTRAN

Table 3  Moments and disposition parameters of VRS, MMC-D, and MMC in the normal muscle. Values were calculated from the outflow patterns in the perfusion experiment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VRS</th>
<th>MMC-D &lt;sub&gt;T-10&lt;/sub&gt;</th>
<th>MMC-D &lt;sub&gt;T-70&lt;/sub&gt;</th>
<th>MMC-D &lt;sub&gt;T-500&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>abc (% of dose - min/ml)</td>
<td>57.9 ± 3.71&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50.8 ± 0.92</td>
<td>49.3 ± 3.11</td>
<td>53.2 ± 4.62</td>
</tr>
<tr>
<td>t (min)</td>
<td>1.37 ± 0.23</td>
<td>1.82 ± 0.09</td>
<td>1.54 ± 2.98</td>
<td>1.59 ± 0.04</td>
</tr>
<tr>
<td>σ&lt;sup&gt;2&lt;/sup&gt; (min&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>2.40 ± 1.23</td>
<td>4.90 ± 0.89</td>
<td>1.68 ± 1.28</td>
<td>3.01 ± 1.09</td>
</tr>
<tr>
<td>F (%)</td>
<td>100</td>
<td>83.1</td>
<td>87.5</td>
<td>92.8</td>
</tr>
<tr>
<td>CL&lt;sub&gt;tot&lt;/sub&gt; (ml/min)</td>
<td>0</td>
<td>0.348</td>
<td>0.233</td>
<td>0.130</td>
</tr>
<tr>
<td>t&lt;sub&gt;s&lt;/sub&gt; (min)</td>
<td>10.7</td>
<td>12.3</td>
<td>22.2</td>
<td>2.88</td>
</tr>
<tr>
<td>V (ml)</td>
<td>2.37</td>
<td>3.71</td>
<td>2.87</td>
<td>2.88</td>
</tr>
<tr>
<td>k</td>
<td>0</td>
<td>0.324</td>
<td>0.276</td>
<td>0.385</td>
</tr>
<tr>
<td>Parameter</td>
<td>MMC-D &lt;sub&gt;T-10&lt;/sub&gt;</td>
<td>MMC-D &lt;sub&gt;T-70&lt;/sub&gt;</td>
<td>MMC-D &lt;sub&gt;T-500&lt;/sub&gt;</td>
<td></td>
</tr>
<tr>
<td>abc (% of dose - min/ml)</td>
<td>49.9 ± 0.99</td>
<td>53.9 ± 3.12</td>
<td>53.5 ± 3.12</td>
<td></td>
</tr>
<tr>
<td>F (%)</td>
<td>85.6</td>
<td>96.4</td>
<td>58.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Results are expressed as the mean ± SD of at least three rabbits.

Table 4  Moments and disposition parameters of VRS and MMC-D<sub>500</sub> (T-500) in the tumor-bearing muscle. Values were calculated from the outflow patterns in the perfusion experiment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>VRS</th>
<th>MMC-D&lt;sub&gt;T-500&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>abc (% of dose - min/ml)</td>
<td>59.9 ± 4.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>46.3 ± 6.20</td>
</tr>
<tr>
<td>t (min)</td>
<td>1.40 ± 0.42</td>
<td>1.60 ± 0.40</td>
</tr>
<tr>
<td>σ&lt;sup&gt;2&lt;/sup&gt; (min&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>1.60 ± 0.67</td>
<td>1.71 ± 1.16</td>
</tr>
<tr>
<td>F (%)</td>
<td>100</td>
<td>75.7</td>
</tr>
<tr>
<td>CL&lt;sub&gt;tot&lt;/sub&gt; (ml/min)</td>
<td>0</td>
<td>0.526</td>
</tr>
<tr>
<td>t&lt;sub&gt;s&lt;/sub&gt; (min)</td>
<td>6.60</td>
<td>6.00</td>
</tr>
<tr>
<td>V (ml)</td>
<td>2.36</td>
<td>3.47</td>
</tr>
<tr>
<td>k</td>
<td>0</td>
<td>0.464</td>
</tr>
</tbody>
</table>

<sup>a</sup> Results are expressed as the mean ± SD of at least three rabbits.

![Fig. 5. Tissue concentration of [14C]MMC-D<sub>500</sub> (T-500) after a 4-h constant infusion in the tumor-bearing muscle. Results are expressed as a percentage of the mean ± SD tissue concentration (g/l) to perfusate concentration (ml/l) of at least three rabbits.](image)

Fig. 6  Tissue concentration of 131I-labeled HSA after a 4-h constant infusion in tumor-bearing muscle. Results are expressed as a percentage of the mean ± SD tissue concentration (g/l) to perfusate concentration (ml/l) of at least three rabbits.

It is well known that tumor tissue has a lower blood flow (20, 21) and higher interstitial pressure than normal tissue (22-24). These conditions are undesirable for delivering drugs to the tumor especially to the central region and become obstacles in cancer chemotherapy (25). The transport of antitumor drugs to tumor tissues has been studied pharmacokinetically in vivo by several investigators (26, 27). However, few studies have been made on the difference in the drug disposition between the tumor and the normal tissue (28, 29).

In the present investigation, the in situ perfusion method was used to evaluate tissue disposition of MMC-Ds because the experimental system becomes simpler, compared with the in vivo experiment using the whole body. Statistical moment analysis was applied, and drug disposition was explained by separating it into the distribution and elimination processes. The present investigation has thus been designed to elucidate the drug disposition characteristics of macromolecules in the tumor by combining these two considerations.

Tumor perfusion has been used for various studies (28, 30-33). Gullino developed a "tissue-isolated" tumor preparation and studied the uptake of DNA in the tumors (31). Jain used the same system to evaluate the drug disposition in the tumors (28). These tumors grew in an artificial condition and their vascular state seemed to be unnatural in part. In the present study, we implanted the tumor in the thigh muscle of rabbits and perfused the whole leg for obtaining information on the vascular permeability of the tumors under a relatively natural condition.

Macromolecules are known to accumulate at the tumor site because of its leaky vasculature and deficit of lymphatic systems.
In this study, MMC-D<sub>oa</sub> (T-500) showed decreased recovery (77.2%) and increased distribution volume in the tumor-bearing muscle compared to normal tissue (Table 4). However, increased blood contamination from other parts of the body and decreased perfusate flow recovery were observed in the tumor-bearing preparation due to the neovascularization of the tumor. This incompleteness of tissue isolation often lowered the reliability of the data. Therefore, it is difficult to conclude from these results that MMC-D<sub>oa</sub> (T-500) is more diffusive in tumor-bearing muscle than in normal muscle.

On the other hand, significant accumulation of [<sup>14</sup>C]MMC-D<sub>oa</sub> (T-500) and <sup>131</sup>I-labeled HSA in the viable region of the tumor was observed in the constant infusion experiment (Figs. 5 and 6). From the total amount of accumulated macromolecules, the accumulation index can be calculated in terms of clearance as follows,

\[ F = (Q - CL)/Q \]

Then

\[ CL = (X(t) - X(0))/(C-t) \]

where \( X(t), CL, C, \) and \( t \) are the amount of the drug transported in the tissue, the disposition clearance, drug concentration of the perfusate, and the perfusion time, respectively. From Equation E, \( CL \) is calculated to be 1.3 \( \mu l/min/g \) tissue for MMC-D<sub>oa</sub> (T-500) and 0.5 \( \mu l/min/g \) tissue for <sup>131</sup>I-labeled HSA. Assuming that the tumor weight is 30 g, drug recovery (\( F \)) is calculated as follows,

\[ F = (Q - CL)/Q \]

where \( Q \) is the flow rate of the perfusate. Using this equation, \( F \) is calculated to be 0.98 and 0.99 for [<sup>14</sup>C]MMC-D<sub>oa</sub> (T-500) and <sup>131</sup>I-labeled HSA, respectively.

Song et al. studied vascular permeability of <sup>125</sup>I-labeled albumin in Walker 256 carcinosarcoma, and found the flow rate of extravasated albumin (identical to \( CL_{oa} \)) to be about 0.1 ml/wet g/h (34). Using this value, \( F \) can be similarly calculated to be 0.97. Therefore, little drug elimination occurs in the bolus injection system, and taking into consideration the experimental error, the elimination of macromolecules cannot be detected.

MMC-D<sub>oa</sub> (T-500) was accumulated in the tumor especially in the viable region when perfused constantly. The central tumor region, which is mostly necrotic, has a poor blood flow (35), and drug delivery to this region appears to be difficult (25). Also in our cases, little [<sup>14</sup>C]MMC-D<sub>oa</sub> (T-500) and <sup>131</sup>I-labeled HSA were observed in this region.

In the normal tissue, macromolecules slowly permeate from the intravascular space to the interstitial space. Then they pass into the lymph vessels but not into the blood. The present results indicated elevated concentrations of MMC-D<sub>oa</sub> and <sup>131</sup>I-labeled HSA in the tumor region, which is mostly necrotic, has a poor blood flow, while MMC is eliminated from the body with a half-life of 24–35 h (Table 1). Consequently, the MMC-D accumulated in the tumor should act as a molecular depot of MMC to successively supply MMC to tumor cells. Direct interaction of MMC-D having a cationic charge with tumor cells (19) should also play a significant role.

In our series of experiments, we have designed various types of mitomycin C-dextran conjugates that are different in molecular size and electric charge (2, 4). These conjugates show highly prolonged retention in the body after systemic administration, while MMC is eliminated from the body with a half-life of 10 min in rats. In general, retention of MMC-Ds increases as their molecular weight increases in both cationic and anionic conjugates. However, the cationic conjugate is accumulated in the liver because of an electrostatic adsorption since this organ has discontinuous capillaries and it can easily make contact with the parenchymal cells. On the other hand, the anionic conjugate, which has no electrostatic interaction with reticuloendothelial organs, is retained in the blood circulation for a considerably long period of time (13, 38). The present results indicate that MMC-Ds would accumulate in the tumor site during constant infusion. Based on these findings, when administered systemically MMC-D<sub>oa</sub> is expected to pass the tumor area via blood circulation at a constant and high concentration and to be lodged there as a molecular depot of MMC.

Superior therapeutic activity of i.v. MMC-D<sub>oa</sub> administration against s.c. implanted Sarcoma 180 in mice (39) seems to be explained by this view.

The present report raises the possibility of using MMC-D for specific delivery of MMC to the tumor site by intraarterial and systemic administration, but the optimum physicochemical property of MMC-D for the delivery to the tumor, must be examined further since it might vary with the type of solid tumor. Accumulation of systematic information on the pharmakinetical characteristics of macromolecular prodrugs would help to improve the drug delivery systems in cancer chemotherapy (12).

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