Disposition of Anticancer Drugs after Bolus Arterial Administration in a Tissue-isolated Tumor Perfusion System

Kazuhiro Ohkouchi, Hiroyoshi Imoto, Yoshinobu Takakura, Mitsuru Hashida, and Hitoshi Sezaki

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ABSTRACT

Disposition characteristics of various anticancer drugs in a tissue-isolated tumor preparation were studied in Walker 256 carcinosarcoma-bearing rats using an in situ single-pass vascular perfusion technique. Three anticancer drugs, 5-fluorouracil, mitomycin C, and Adriamycin, and two lipophilic prodrugs of mitomycin C were tested in the tumor preparation perfused with Tyrode's solution containing 4.7% bovine serum albumin. After bolus arterial injection of test drugs, their outflow concentration-time curves were analyzed based on statistical moment theory. In each tumor preparation, the injection of drug was paired with that of vascular reference substance, Evans' blue-labeled bovine serum albumin, and disposition parameters of the drug were corrected with those of vascular reference substance. From the mean transit time values of vascular reference substance, the average vascular volume of the tumor preparation was calculated to be 0.063 ml/g, which decreased with tumor growth. All drugs showed significant extraction by the tumor tissue, depending on their physicochemical properties. Distribution volumes of tested drugs were from 1.53 to 3.33 times larger than the vascular volume. Calculated intrinsic clearance values for the protein-unbound fractions increased as the lipophilicity of the drug increased. The potential increase in tumor uptake was observed in lipophilic prodrugs of mitomycin C. The present experimental system is thus suggested to be useful for analyzing drug disposition in tumor tissue.

INTRODUCTION

In cancer chemotherapy, it is necessary to concentrate cytotoxicity of an anticancer agent to the tumor and to minimize the exposure of other normal tissues to it. To accomplish this, we have reported several efforts, such as alternation of the route of administration or dosing schedule and physical or chemical transformation of drugs. In our series of investigations we have developed lipophilic and polymeric prodrugs of MMC and examined their physicochemical, pharmacodynamic, and pharmacokinetic properties (1, 2). These studies demonstrated that the in vivo disposition of MMC could be controlled by choosing physicochemical characteristics of carrier moiety in prodrugs.

The intraarterial administration of anticancer drugs including the chemoembolization approach has become a matter of great interest (3, 4). However, little information has been obtained on disposition profiles of anticancer drugs from the intravascular space to tumor tissues. In a series of investigations, we have established an experimental system for analyzing drug disposition in a local tissue based on the statistical moment theory (5). The disposition of various drugs in the rat liver and in the normal and tumor-bearing muscles of the rabbit (6, 7) has been studied with this system, and its relation with the physicochemical properties of drugs was elucidated.

In the present study, disposition characteristics of anticancer drugs and prodrugs were studied in a tissue-isolated Walker 256 tumor preparation in the rat.

MATERIALS AND METHODS

Chemicals. MMC, ADR, and FU were kindly supplied by Kyowa Hakko Kogyo Co., Tokyo, Japan. Propyl-MMC and pentyl-MMC were synthesized as reported previously (8–10). 5-Fluoro[2-14C]uracil was purchased from CEA, Cedex, France. All other chemicals were of reagent grade and obtained commercially.

Animals and Tumors. SLC female Wistar rats weighing 100 to 120 g were obtained from Shizuoka Agricultural Association for Laboratory Animals, Shizuoka, Japan. Walker 256 sarcoma was kindly supplied by Shionogi & Co., Osaka, Japan, and maintained by s.c. inoculation to another rat every 2 wk.

Preparation of Tissue-isolated Tumors. Ovarian tissue-isolated tumors were prepared according to the method of Gullino and Grantham (11). Rats were anesthetized by i.p. injection of pentobarbital. The ovary, uterus, and mesovarium were pulled from the peritoneal cavity through a 1-cm incision in the skin and muscle wall of the left lumbar region. The uterine horn was ligated distally to the origin of the ovary, and the mesovarium and ovary were ligated proximally to the ovarian artery and vein. Three blocks of minced Walker 256 sarcoma were inoculated in the adipose tissue around the ovary, and the inoculated adipose tissue was wrapped with Sealon film (Fuji Film Co., Tokyo, Japan) to separate from other tissue. The Sealon film sheet was folded, and its margins were melted to form a sealed envelope. The tumor-inoculated tissue was placed between the cutaneous and muscle wall of the left lumbar region. The tumor mass wasperfused with Tyrode's solution (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 BSA (Fraction V) at a concentration of 4.7% (w/v). The same medium was used for drug injection. The concentrations of injection solution (0.1 ml) were 10 mg/ml for FU (1 μCi/ml for 5-fluoro[2-14C]uracil; 1 mg/ml for EB, MMC, and pentyl-MMC; and 0.1 mg/ml for propyl-MMC. The perfusate was gassed with 95% O2-5% CO2 and maintained at 37°C. Evans' blue/BSA was used as a VRS and applied to the tumor preparation prior to the drug injection for obtaining basic information about each tumor tested.

Sampling and Assay. The outflowing perfusate was collected into previously tared tubes at appropriate time intervals (at first 5 s, subsequently 10 to 15 s). The sample volume was calculated from the gain in weight of the tube by assuming a density of the outflow perfusate to be 1.0. The sample was subjected to assay after an appropriate dilution with the perfusate buffer. The concentration was determined spectrophotometrically at 620 nm for EB/BSA and at 479 nm for ADR.
DISPOSITION OF ANTI-CANCER DRUGS

Rat
Perfusote

Fig. 1. Perfusion system of tissue-isolated tumor preparation.

Table 1 Derivation of the disposition parameters from moments

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extent</td>
<td>( R_i = \text{auc}_i / Q_i )</td>
</tr>
<tr>
<td>Amount of recovery</td>
<td>( F_i = \text{auc}<em>i / \text{auc}</em>{VRS} )</td>
</tr>
<tr>
<td>Recovery ratio</td>
<td>( t_{el} = t_i / E_i )</td>
</tr>
<tr>
<td>Rate</td>
<td>( CL_{int.,} = V_i / t_{el} )</td>
</tr>
<tr>
<td>Mean elimination time</td>
<td></td>
</tr>
<tr>
<td>Clearance</td>
<td></td>
</tr>
<tr>
<td>Intrinsic clearance</td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td></td>
</tr>
<tr>
<td>Extent</td>
<td>( V_i = Q_i / F_i )</td>
</tr>
<tr>
<td>Distribution volume</td>
<td>( k_i = (t_i/F_i) / \text{auc}_i - 1 )</td>
</tr>
<tr>
<td>Tissue distribution ratio</td>
<td></td>
</tr>
</tbody>
</table>

MMC, propyl-MMC, and pentyl-MMC were assayed by a high-performance liquid chromatography system (Trizolet; Jasco, Tokyo, Japan) equipped with a variable wavelength UV detector (UVIDEC 100-11; Jasco) at 365 nm. The stationary phase used was a Cosmolsil 5C18 packed column (4.6 x 150 mm; Nacalai Tesque, Kyoto, Japan), and a short column packed with Lichrosorb RP-2 (B. Merck, AG, Darmstadt, West Germany) was used to guard the main column. Methanol:water mixtures (35:65 for MMC and 60:40 for propyl-MMC and pentyl-MMC) were used as the mobile phase with a flow rate of 0.8 ml/min. The precise limit of sensitivity was 0.1% of dose/ml. For the determination of FU, 5 ml of scintillation medium (Cleasol; Nacalai Tesque, Kyoto, Japan) were added to the sample, and \(^{14}\)C radioactivity was measured in a liquid scintillation system (LSC 900; Aloka, Tokyo, Japan).

Pharmacokinetic Analysis of Outflow Pattern. Data analysis was carried out based on the statistical moment concept. The detailed theoretical description of this analysis was given previously (1,7). The 0th and first moment parameters (moments) for output response are defined as follows

\[
\text{auc}_i = \int_{0}^{t} C \, dt
\]

\[
t_i = \int_{0}^{t} t \times C \, dt / \text{auc}_i
\]

where \( t \) is time and \( C \) is the concentration of a test substance normalized by injection dose with a dimension of "% of dose/ml." \( \text{auc}_i \) and \( t_i \) are the area under the concentration-time curve and the mean transit time, respectively. The moments were calculated by numeral integration using a linear trapezoidal formula from the outflow concentration-time curve. All \( t_i \) values were corrected by subtracting the time corresponding to the transit through afferent and efferent tubes.

In the next step, distribution parameters were calculated from the moments as summarized in Table 1 (5). Each parameter is represented as: \( R_i \), recovery percentage of drug corrected by outflow flow; \( F_i \), recovery ratio of drug to that of VRS; \( t_{el} \), mean time necessary for elimination of the drug from the perfusate compartment; \( CL_{int.,} \), intrinsic clearance; \( V_i \), distribution volume; and \( k_i \), ratio of amount of drug in the extravascular tissue to that in the perfusate.

Determination of Partition Coefficient of Drug. The partition coefficient of the drug was determined as a ratio of the drug concentration between 20 ml of octanol and water after mixing and equilibration at 37°C for 48 h.

Determination of Protein Binding. The nonbinding percentage of drug with BSA in the perfusate (fu) was determined by the centrifugal ultrafiltration method (Ultracent-30; Tosoh Co., Tokyo, Japan). The intrinsic clearance for the free fraction of drug (CL_{int.,fu}) was calculated as \( CL_{int.,fu} = CL_{int.,} / fu \).

Angiography of Tumor Tissue. Megalmine diatrizoate (Sigma) was solvated in the perfusate as 65% (w/v) and continuously injected from the artery during soft (low kVp) X-ray examination.

Determination of True Vascular Volume. The vascular volume of the present preparation calculated as the \( V_i \) value of VRS includes not only vascular space in the tumor mass but also that of appendicular vascular matter between the tumor mass and the cannulation points. The latter was determined from the residual perfusate volume in there at the end of the perfusion experiment and subtracted from the \( V_i \) value of VRS to estimate the true vascular volume of the tumor.

RESULTS

Tumor Perfusion. An angiogram of the tumor preparation confirmed the establishment of the isolated perfusion system for the Walker 256 tumor mass (Fig. 2). The mean recovery of the perfusate from the venous cannula was about 58.1% in this system.

Outflow Patterns in the Tumor Tissue. Fig. 3 shows the typical concentration-time curves of FU (A) and pentyl-MMC (B), together with those of VRS in the same tumor preparations.

Fig. 2. Angiogram of tissue-isolated tumor preparation. t, tumor tissue; a, arterial inflow side; v, venous outflow side.
The concentration of each test drug was lower in the initial phase and higher in the later phase than those of VRS. Especially, the FU concentration showed a gentle decline in the first 100 s after injection when it overcame that of VRS. The outflow patterns of ADR, MMC, and propyl-MMC were basically similar to that of pentyl-MMC (data not shown).

Moments and Disposition Parameters. Tables 2 and 3 summarize the moments and the disposition parameters obtained in this system, respectively. All test compounds showed smaller auc, and longer $t_1/2$ than those of VRS, which were obtained by preceding injection in the same tumor preparation (Table 2). Disposition parameters representing distribution, elimination, and clearance of drugs varied depending on their physicochemical properties. These results show that the value of CL$_{int,v}$ increased as the lipophilicity of drugs increased.

**DISCUSSION**

In general, drugs are rapidly extracted from the blood by several organs such as the liver and kidney, and these conditions are considered to be disadvantageous for systemic drug delivery to the tumor tissue in cancer chemotherapy. Therefore, local administration modalities such as intraarterial infusion are adopted to enhance the drug exposure to tumor tissue. To improve the efficiency of intraarterial infusion, several approaches have been reported such as vascular obstruction, use of vasoactive agents (12, 13), utilization of oily medium (14), and chemoembolization using microcapsules (15). Chemical modification of drugs into a lipophilic or a polymeric form also would be a promising strategy.

The purpose of this study was to estimate the pharmacokinetic behavior of anticancer drugs and their prodrugs in a tissue-isolated tumor preparation using an analytical method for local drug disposition based on moment theory. A tissue-isolated preparation has been applied to elucidate physiological properties of tumors such as blood flow, vascular volume, and oxygen and glucose consumption (16), and more recently, to study the effect of hyperglycemia on hemodynamics and pH responses in the tumor (17). In spite of these basic research investigations, however, few studies have been done on drug disposition using this method. In a previous paper, we have investigated the disposition of MMC and its polymeric prodrugs in the single-pass perfusion system of the tumor-bearing muscle of rabbits (2). From this study, however, only mixed information for both the normal and tumor tissues was obtained. In the present study, disposition characteristics of drugs in the tumor tissue can be independently analyzed in a tissue-isolated tumor preparation. Using this tissue-isolated tumor preparation, we can manipulate some experimental conditions, such as perfusate composition, and appreciate the effects of perfusion rate, injection medium, hyperthermia, and so on.

In the present study, drugs and prodrugs were introduced as a pulse (7.5 s) function from the arterial side, and venous outflow patterns were analyzed by statistical moment theory. Although tumor tissue was shown to be perfused and was isolated from other tissues by the present experimental procedure, as shown in Fig. 2, the mean outflow recovery was only about 58.1%. In contrast to the liver and muscle perfusion experiments which gave almost 100% outflow recovery (2, 6), it was difficult to satisfactorily recover the flow from the venous side in this tumor perfusion system, mainly due to leakage from the unligatable venous vessels. In the present analysis, however, the disposition parameters can be estimated in spite of low outflow recovery, because when plural processes exist in a perfusion circuit after the passage through the tumor, the outflow concentration should be the same in each pathway. Assuming that all of perfusate passed through the tumor tissue and then loss of perfusion fluid occurred after this, the disposition parameters could be calculated from the outflow concentration-time curve and inflow rate ($Q$). The fact that there was no permeate in the arterial side in Fig. 2 and other experiments.
values for the vascular volume of rabbit muscle (5) and rat liver (21) by the same procedure.

The vascular volume of the present tissue-isolated Walker 256 carcinoma in the weight range of 1 to 20 g was shown to be represented, versus total tumor weight, by the equation, vascular volume (ml/g) = $-0.00494 \times$ tumor weight (g) + 0.107. That is the vascular volume content decreased with an increase in tumor weight, and the vascular volume content of an average size tumor (8.71 g) was 0.070 ml/g (Fig. 4). Song and Levitt (22) reported that the average vascular volume of s.c. implanted Walker 256 carcinoma was 0.079 ml/g and that this volume decreased linearly with tumor size, using $^{51}$Cr-labeled erythrocytes. Gulino and Grantham (23) found that the vascular volume of tissue-isolated Walker 256 carcinoma determined with Dextran 500 ($M_f$, 375,000) was almost constant at the value of about 0.1 ml/g when the tumors were small (4 to 12 g), but no correlation was observed in larger tumors. The present results are essentially in good agreement with these data.

In this study, disposition parameters for drugs representing distribution, elimination, and intrinsic clearance were calculated from the primary moment parameters (Table 3). To compensate for the variance among tumor preparations, parameters for each drug were determined together with those of VRS in the same preparation.

FU showed peculiar disposition characteristics among the tested compounds, i.e., a large $V_t$, low extraction (large $\text{fu}$), and large $k_i$. These results suggest that FU easily moves to the extravascular space during the passage of drug solution, but most of it returned to the intravascular space in the following step. In MMC and ADR, 34 and 19% of administered dose were taken up by the tumor, respectively, suggesting their effective delivery to the tumor by intraarterial administration. Two types of lipophilic prodrugs of MMC showed a little larger $V_t$ and smaller $F_i$ than the parent drug, suggesting an enhanced uptake by the tumor. It was difficult, however, to discuss the disposition characteristics of lipophilic prodrugs from these data, since the effect of protein binding cannot be neglected.

In order to examine true disposition characteristics, intrinsic clearance corrected by the free fraction (CL$_{int, fu}$) of each compound was calculated (Table 4). The correlation between lipophilicity and protein binding indicates that the lipophilic drugs are likely to bind albumin (24). It was clear that the CL$_{int, fu}$ increased with an increase in lipophilicity and that pentyl-MMC

### Table 3

<table>
<thead>
<tr>
<th>R$_t$ (%)</th>
<th>$F_i$</th>
<th>$t_{fu}$ (s)</th>
<th>CL$_{int, fu}$ (ml/min/g)</th>
<th>$V_t$(ml/g)</th>
<th>$k_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>EB/BSA</td>
<td>97.5 ± 2.9*</td>
<td>-</td>
<td>0.0987 ± 0.0219</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>FU</td>
<td>82.9 ± 6.0</td>
<td>0.851 ± 0.054</td>
<td>827 ± 184</td>
<td>0.0247 ± 0.0091</td>
<td>0.329 ± 0.090</td>
</tr>
<tr>
<td>MMC</td>
<td>106.5 ± 6.8</td>
<td>1</td>
<td>-</td>
<td>0.0917 ± 0.0447</td>
<td>0</td>
</tr>
<tr>
<td>ADR</td>
<td>70.4 ± 4.9</td>
<td>0.664 ± 0.084</td>
<td>230 ± 106</td>
<td>0.0484 ± 0.0120</td>
<td>0.172 ± 0.037</td>
</tr>
<tr>
<td>Propyl-MMC</td>
<td>95.8 ± 5.2</td>
<td>1</td>
<td>-</td>
<td>0.0792 ± 0.0321</td>
<td>0</td>
</tr>
<tr>
<td>Pentyl-MMC</td>
<td>78.1 ± 15.1</td>
<td>0.812 ± 0.118</td>
<td>308 ± 221</td>
<td>0.0255 ± 0.0043</td>
<td>0.121 ± 0.071</td>
</tr>
<tr>
<td>EB/BSA</td>
<td>105.3 ± 9.5</td>
<td>1</td>
<td>-</td>
<td>0.0949 ± 0.085</td>
<td>0</td>
</tr>
<tr>
<td>Propyl-MMC</td>
<td>61.7 ± 19.6</td>
<td>0.587 ± 0.128</td>
<td>154 ± 100</td>
<td>0.0875 ± 0.0769</td>
<td>0.175 ± 0.157</td>
</tr>
<tr>
<td>EB/BSA</td>
<td>102.6 ± 8.9</td>
<td>1</td>
<td>-</td>
<td>0.0923 ± 0.014</td>
<td>0</td>
</tr>
<tr>
<td>Pentyl-MMC</td>
<td>57.8 ± 8.1</td>
<td>0.572 ± 0.130</td>
<td>160 ± 7.8</td>
<td>0.0744 ± 0.0095</td>
<td>0.197 ± 0.020</td>
</tr>
</tbody>
</table>

* Mean ± SD.

### Table 4

<table>
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<tr>
<th>MF</th>
<th>PC$_{int}$*</th>
<th>fu</th>
<th>CL$_{int, fu}$ (ml/min/g)</th>
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<tr>
<td>FU</td>
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<td>0.152</td>
<td>0.9813</td>
</tr>
<tr>
<td>MMC</td>
<td>334</td>
<td>0.360</td>
<td>0.8935</td>
</tr>
<tr>
<td>ADR</td>
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<td>0.463</td>
<td>0.4915</td>
</tr>
<tr>
<td>Propyl-MMC</td>
<td>420</td>
<td>32.7</td>
<td>0.691</td>
</tr>
<tr>
<td>Pentyl-MMC</td>
<td>488</td>
<td>279</td>
<td>0.160</td>
</tr>
</tbody>
</table>

* PC$_{int}$ partition coefficient of drug between octanol and water; fu, nonbinding percentage of drug with BSA.

* Mean ± SD.

### Figure 4

Relation between tumor weight and intravascular volume. The vascular volume was shown to be represented against tumor weight by the following equation in weight area between 1 and 20 g: Intravascular volume (ml/g) = $-0.00494 \times$ tumor weight (g) + 0.107.

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with dye (EB/BSA) perfusion support these assumptions. The validity of this analysis was also confirmed by the result that the amount of recovery ($R_t$) of VRS was almost 100% in all groups (Table 3).

Tumor tissues are characterized by enhanced vascular permeability to macromolecules (18, 19). However, extravasation of EB/BSA would be negligible during a single passage of tumor tissue; tumor uptake rates of BSA were calculated to be 30 µl/h/g for rabbit VX2 carcinoma and 14.2 µl/h/g for mouse S-180 carcinosarcoma (20) in previous studies. Therefore, the $V_t$ of EB/BSA can be considered to correspond to the vascular volume of tumor tissue. We have already obtained reasonable

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had the highest CL_{int,inc} value. This indicates that chemical transformation of a drug into a lipophilic form is desirable for the delivery to the tumor from the vascular space. However, the difference in the net uptake of the drug by the tumor (E_t) was not so large, since the lipophilicity also increased the protein-bound fraction which basically did not contribute to the tumor uptake. It is concluded from these results that intraarterial infusion of anticancer drugs especially lipophilic prodrugs would show higher uptake by the tumor when applied in a binder (albumin)-free formulation.

Prodrugs must be regenerated to the parent drug before exhibiting therapeutic activities. Regeneration processes of the present MMC prodrugs have been analyzed previously in the rabbit muscle through Laplace transformation of the convolution function for them (7). It was confirmed that lipophilic derivation produced a larger tissue distribution and that rapid regeneration of the drug from the prodrug resulted in a large local bioavailability of the parent drug.

Thus, the utility of a novel experimental system for analyzing the drug disposition in a tissue-isolated tumor preparation based on the statistical moment concept was demonstrated. The present system also can be applied for the evaluation of various drug delivery approaches, such as utilization of a macromolecular or spherical (liposome, microsphere) carrier system and combination chemotherapy with vasoactive agents and hyperthermia, etc. Especially, macromolecular carrier systems seem to be of interest since macromolecules have some advantageous features for tumor targeting (25).

REFERENCES

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