Pharmacodynamics of Mitomycin C in Cultured Human Bladder Tumors

Thomas D. Schmittgen, M. Guillaume Wientjes, Robert A. Badalament, and Jessie L.-S. Au

College of Pharmacy [T. D. S., J. L.-S. A.] and Division of Urology [M. G. W., R. A. B.], The Ohio State University, Columbus, Ohio 43210

ABSTRACT

The effects of mitomycin C (MMC) concentration and exposure time on the inhibition of tumor cell labeling index (LI) were determined by incorporation of [3H]thymidine and autoradiography. All tumors responded to MMC. However, the sensitivity varied significantly between tumors. At a 2-h exposure, the concentrations required for 50 and 90% inhibition (IC50 and IC90) ranged from 0.237 to 14.9 and 2.76 to 74.5 μg/ml, respectively. There was an inverse correlation between MMC activity and tumor LI; the IC values were higher for the more rapidly proliferating tumors. Exposure time had a pronounced effect on MMC activity. Shortening the exposure time from 2 to 0.5 h increased the IC3 fold, while prolonging the exposure time from 2 to 24 h decreased the IC6-fold. To determine the minimum concentration and exposure time necessary to reduce tumor LI by 90%, the data for 6 tumors were computer analyzed. The analysis showed that, on average, a 2.5-h exposure of 3 μg/ml was needed for 50% inhibition and a 7-h exposure of 8 μg/ml was needed for 90% inhibition. A comparison of the IC values of MMC determined in this study with the literature values determined using monolayer and spheroid cultures of established human bladder tumor cell lines showed that the drug activity in cultured tumor fragments ranged from 7- to 5300-fold lower than that in established cell lines. In summary, our data demonstrate a heterogeneity in the response of bladder tumors from individual patients to MMC, a decreased sensitivity to MMC with increasing tumor proliferation, and that drug concentration and exposure time are critical determinants of MMC activity.

INTRODUCTION

Cancer of the urinary bladder is the fourth most prevalent type of malignant cancer in men and the sixth in women (1). More than 50,000 new cases will be diagnosed in 1991, and of these 75–85% will be superficial (1, 2). Between 50 and 80% of the patients will develop recurrent disease and in 10–15% of the cases the recurrent disease will be accompanied by grade and/or stage progression (3). Therapy for superficial bladder cancer consists of transurethral resection. In the high risk patients, this is followed by intravesical administration of agents such as MMC, doxorubicin, or thiopeta. The goal of intravesical therapy is to eradicate any existing tumor and to reduce the likelihood of tumor recurrence. The response to intravesical therapy has been variable and unpredictable. In a review of 9 studies, the decrease in the frequency of recurrence following transurethral resection and intravesical therapy with MMC, doxorubicin, or thiopeta compared to resection alone ranged from 2 to 70% (4). The cause of the highly variable response rate is unknown. In addition, the T1 tumors respond more favorably to intravesical MMC therapy than the T2 tumors (5). It is not known whether this is due to a difference in the tumor sensitivity to the drug or the differences in drug concentrations at the two sites, i.e., urothelium for the T1 tumors and lamina propria for the T2 tumors.

A number of factors including the dose, effective drug concentration, and dwelling time of instillation may determine the therapeutic efficacy. At present, the dose and dwelling time of intravesical therapy are primarily based on empiricism. For example, a commonly used therapy consists of 20–40 mg of MMC dissolved in 40 ml of water and instilled for 2 h. While the empirical regimen has proven to be effective in 10–50% of patients (3, 6–8), a better understanding of the relationship among the drug concentration, dwelling time, and effect may allow further improvement of the treatment regimen. The pharmacological basis of the variable and incomplete response and of the different tumor sensitivity is of interest and may aid in defining new treatment strategies for nonresponding tumors. Furthermore, identification of prognostic indicators of drug sensitivity such as the tumor pathology can aid in the selection of the most effective agents.

To understand the dose-effect relationship in patients, pharmacokinetic data describing the drug absorption, distribution, and metabolism and pharmacodynamic data describing the drug concentration-exposure time effect relationship at the target site are necessary. Although there is a large data base of human pharmacokinetic data, the pharmacodynamics of anticancer agents in preclinical and clinical models are poorly understood (9). Herr (4) discussed the lack of pharmacodynamic data concerning intravesical therapy for the treatment of superficial bladder cancer and the importance of these data.

Ideally, pharmacodynamics of intravesical therapy should be studied using an in vivo model of human bladder cancer with the tumor located in the bladder. The study of pharmacodynamics in patients is technically difficult because of the inability to measure both the drug concentration at the target site and the drug effect. An s.c. implant of human xenografts in nude mice would not be appropriate because it does not permit direct contact of the tumor with the drug instilled in the bladder. Bladder tumors may be induced in rodents with chemical carcinogens (10). However, the chemosensitivity of a carcinogen-induced animal tumor may not reflect that of a spontaneous human tumor. Furthermore, a complete pharmacodynamic study in which different drug concentrations and different exposure times were used would require a large number of animals. Assessment of drug effect in a deep tissue tumor presents additional technical difficulty. As an alternative, we used an in vitro model to study the pharmacodynamics of intravesical therapy in human bladder cancer. Important aspects of treatment which could be addressed by an in vitro pharmacodynamic study include (a) determination of drug concentrations and exposure time necessary to produce a desired effect, (b) predic-
tion of drug sensitivity for individual patients, and (c) examination of the pharmacological and biological basis for drug sensitivity and resistance.

Continuous human bladder cell lines have been used for chemosensitivity studies (11-16). Shortcomings of using cell lines include the lack of tumor heterogeneity and the inability to predict drug effect in a clinical situation. We (17) and Perrapato et al. (18) described a method to culture fragments of freshly isolated human bladder tumors on collagen gels and showed that these tumors can be maintained in their native state in culture for up to 180 days without loss of the original tumor morphology. This method is similar to that described by Hoffman et al. (19, 20) for culturing other solid human tumors. The advantages of this system include the ease and high success rate of human tissue culture, maintenance of three-dimensional tissue architecture, cell-cell interactions, and cell heterogeneity. For evaluation of drug activity, drug penetration in this multicell layer system is more in vivo like than that in the monolayer systems.

The goal of this study was to investigate the relationship among drug concentration, exposure time, and cytotoxic effect of MMC in bladder tumors from individual patients under in vitro conditions. We report here the intersubject variability in the tumor sensitivity to MMC, the relationship of tumor response with drug concentration and exposure time, and the relationship between drug sensitivity and tumor proliferative activity.

MATERIALS AND METHODS

Chemicals and Supplies. Sterile pigskin collagen (Spongostan standard) was purchased from Health Designs Industries (Rochester, NY), cefotaxime sodium from Hoechst-Roussel (Somerville, NJ), NTB-2 nuclear track emulsion from Eastman Kodak Co. (Rochester, NY), and [methy1-3H]dThd from ICN Biomedicals (Irvine, CA). MMC was a gift from Bristol-Myers Co. (Wallington, CT). All other reagents, tissue culture medium, and supplies were purchased from Gibco Laboratories (Grand Island, NY). All of the reagents were of the highest quality and were used as received.

Tumor Specimens. Primary human bladder cancer tumors were obtained through the Cooperative Human Tissue Network at the Ohio State University. Tumor stage (depth of penetration) was determined according to the American Joint Committee on Cancer (21) and tumor stage (depth of penetration) was determined by pathological biopsy on the surgical specimens. Ploidy was determined as diploid, aneuploid, or non-ploidy. Using the criteria of Ploidy and Cytometry. Culture of human bladder tumors in collagen gels and tissue culture. Tumor morphology. This method is similar to that described by Hoffman et al. (19, 20) for culturing other solid human tumors. The advantages of this system include the ease and high success rate of human tissue culture, maintenance of three-dimensional tissue architecture, cell-cell interactions, and cell heterogeneity. For evaluation of drug activity, drug penetration in this multicell layer system is more in vivo like than that in the monolayer systems.

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Tumor Specimens. Primary human bladder cancer tumors were obtained through the Cooperative Human Tissue Network at the Ohio State University. Tumor stage (depth of penetration) was determined according to the American Joint Committee on Cancer (21) and tumor grade (degree of differentiation) according to a modified Armed Forces Institute of Pathology schema (22). Patient and tumor data are listed in Table 1. All bladder tumor specimens were obtained by transurethral resection or cystectomy and placed immediately in Hanks' balanced salt solution. The specimens were prepared for culture within 2-4 h post-surgery. Twelve of the 14 patients had no prior history of drug treatment. One patient had prior exposure to intravesical MMC and one patient to systemic MVAC. The previous treatments were administered at least 1 month prior to the present study. No drug treatment was administered prior to surgery.

Tissue Culture. Tumor specimens were cultured as previously described (17). Briefly, the necrotic portions of the tumor were trimmed off and the nonnecrotic portions were cut into 1-mm3 fragments. This corresponded to an average of 130 cells in a cross-section, as determined by microscopic analysis of a randomly selected portion of 54 samples. The fragments were mixed to ascertain randomization. Four to 5 tumor fragments were placed on a 1-cm2 piece of collagen gel and cultured in 6-well plates in a humidified atmosphere of 95% air-5% CO2 at 37°C. The culture medium consisted of Eagle's minimal essential medium supplemented with 9% heat-inactivated fetal bovine serum, 0.1 mm nonessential amino acids, and antibiotics, gentamicin (100 μg/ml) and cefotaxime sodium (95 μg/ml). The pH of the medium was 7.4. The proliferative activity of the tumors was determined by [3H]dThd labeling and autoradiography. Cultures were incubated with medium containing [3H]dThd with a specific activity of 60 Ci/mmol, at a concentration of 4 μCi/ml for 4 days. Afterward, tissues were fixed in 10% buffered formalin overnight, dehydrated, and embedded into paraffin blocks. The blocks were cut into 4-μm sections using a microtome and the sections were fixed onto microscope slides. The slides were deparaffinized, hydrated, and stained with hematoxylin and eosin.

Flow Cytometry. DNA ploidy was determined by flow cytometric analysis of the paraffin-embedded, primary, uncultured tumors. The methodology was similar to that reported previously (23). Briefly, three 50-μm sections were sectioned from 2 representative tissue blocks of the normal and malignant paraffin-embedded tissues from a patient. The normal tissue was used as the internal standard for the aneuploid tumors. The samples were deparaffinized and rehydrated, digested with pepsin, and filtered. The resulting suspension (4 x 106 to 2 x 106 cells) was incubated with 0.1% Triton X-100 for 3 min, followed by ribonuclease digestion and staining with propidium iodide. The stained nuclei were measured on an EPICS Profile flow cytometer (Coulter Corp., Hialeah, FL). An aneuploid tumor population was defined as one having at least 2 separate G0/G1 peaks (24).

Pharmacodynamic Studies. Drug effect was quantitated as the inhibition of the LI. Cultures were maintained for a minimum of 4 days prior to drug exposure. The tumor explants were exposed to MMC for 2 h. This is equivalent to the duration of drug instillation for intravesical therapy in patients. Four to 5 concentrations of MMC were used per experiment, depending on tissue availability. Triplicate or quadruplicate wells were used for each drug concentration. Each well contained 4-5 x 105 cells or 0.5 x 106 cells for each drug concentration. Each well contained 4-5 x 105 cells or 0.5 x 106 cells for each drug concentration. Each well contained 4-5 x 105 cells or 0.5 x 106 cells for each drug concentration. Each well contained 4-5 x 105 cells or 0.5 x 106 cells for each drug concentration. Each well contained 4-5 x 105 cells or 0.5 x 106 cells for each drug concentration.

Table 1 Patient and tumor data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Grade</th>
<th>Stage</th>
<th>Ploidy</th>
<th>Size (cm³)</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Surgery performed</th>
<th>Previous drug treatment</th>
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* Intravesical MMC was given 1 month prior to this study.
* Intravenous treatment was given 6 months prior to this study.

3850
for each drug concentration. In a pilot study, we determined that the collagen gel retained about 0.8 ml of fluid. In a total volume of 4 ml/well, this corresponded to a 20% dilution by residual media in the gel. The MMC solutions were prepared so that they corrected for this dilution effect and gave the desired concentration. Following exposure, the drug-containing medium was removed and the gel was rinsed 3 times with 4 ml of drug-free medium. [3H]dThd was immediately added and incubated for 4 days. Samples were then processed for autoradiography. To determine LI, microscopic sections were quantitated at the most active area as previously discussed (17). This method standardized the selection procedure for evaluation of different samples and gave a conservative estimate of drug effect rather than an overestimation. LI was defined as the number of labeled nuclei divided by the total number of nuclei in the field, at x400 magnification. To test the effect of altered exposure time on MMC activity, tumor explants were exposed to MMC for periods of 0.5, 2, 4, 8 and 24 h. About 1 g of tumor was needed for a complete pharmacodynamic experiment with 5 different exposure times.

Data Analysis. The concentration-effect relationship was analyzed by computer fitting the experimental data to one of the following equations.

\[ E = 100\% - \frac{E_{\text{max}} \cdot C^a}{IC_{50} + C^a} \]  

(A)

\[ E = S \cdot \log C + I \]  

(B)

\[ C^* \times T = k \]  

(C)

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(C)
PHARMACODYNAMICS OF MMC IN BLADDER CANCER

Fig. 1. MMC concentration-effect relationship. A bladder tumor (patient 9) was exposed to various MMC concentrations for 2 h. The LI was determined and expressed as a percentage of control. Points, mean experimental data; bars, SEM; line, computer-fitted relationship according to Equation A.

Fig. 2. MMC inhibitory concentration versus tumor proliferate index. The IC_{50} of MMC (2-h exposure) was plotted against the LI of untreated controls (n = 14). The correlation coefficient obtained by linear regression was 0.748 (P < 0.001).

activity between 3 and 14 days; after 14 days the LI decreased (17). One tumor was cultured for 19 days and one for 22 days. Their LI and IC_{50} were lower than the mean value. It is not clear whether the longer culture time had affected the LI and IC_{50} in these two tumors.

Drug effect is expressed as a percentage of control. Fig. 1 shows a representative concentration-effect relationship. The curve was sigmoidal in shape and had a maximum effect approaching 100% inhibition. The data were computer fitted to Equation A or B, and estimates for n, E_{max}, IC_{50}, and IC_{90} were obtained. Table 2 lists the results of 14 patients. The IC_{50} values of tumors from the 2 patients with prior MVAC or MMC treatment were not different from the group results. The IC_{50} ranged from 0.237 to 14.9 μg/ml and the IC_{90} values were 2.76 to 74.5 μg/ml, respectively. Thus, a trend of decreasing MMC activity with increasing tumor invasiveness was observed, but the correlation was not statistically significant. The tumors were separated into 2 groups according to ploidy, i.e., diploid and aneuploid. The IC_{50} was 2.37 ± 2.06 (n = 5) for the diploid tumors and 5.23 ± 4.40 (n = 9) for the aneuploid tumors. The difference was not statistically significant (P = 0.21).

Effect of Exposure Time on MMC Pharmacodynamics. The effect of drug exposure time on the inhibition of tumor cell proliferation was studied following exposure times of 0.5, 2, 4, 8, and 24 h. It is noted that the analysis used only the initial drug concentration and assumed no significant loss of drug over the exposure time of 0.5 and 24 h. The concentration-effect relationship for a tumor exposed for periods of 0.5, 4, and 8 h shows a 27-fold difference in tumor sensitivity for exposure times of 0.5 and 8 h (Fig. 3A). The data for 14 tumors produce an equal effect in the more rapidly proliferating tumors.

We previously showed a correlation between the LI of human bladder tumors (untreated) and tumor grade and stage (17). In the present study, a similar correlation was observed. The LI increased with increasing tumor grade (P < 0.05) and stage (P < 0.10). We further compared MMC activity by tumor grade and stage. There was no correlation between the IC_{50} and tumor grade (P = 0.66). For the T_{1} and T_{2} tumors, the IC_{50} values were 3.5 ± 3.3 (mean ± SD), 2.3 ± 2.2, and 7.0 ± 4.8 μg/ml and the IC_{90} values were 16.2 ± 4.2, 24.6 ± 26.6, and 42.9 ± 26.1 μg/ml, respectively. Thus, a trend of decreasing MMC activity with increasing tumor invasiveness was observed, but the correlation was not statistically significant. The tumors were separated into 2 groups according to ploidy, i.e., diploid and aneuploid. The IC_{50} was 2.37 ± 2.06 (n = 5) for the diploid tumors and 5.23 ± 4.40 (n = 9) for the aneuploid tumors. The difference was not statistically significant (P = 0.21).

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Fig. 3. Effect of exposure time on MMC activity. A, effect of 0.5-, 4-, and 8-h exposure on the concentration-effect relationship (patient 14). Symbols, experimental data: lines, nonlinear least-squares fit according to Equation A. B, mean IC_{50} and IC_{90} values from the patient group studied at exposure times of 0.5, 2, 4, 8, and 24 h. The sample size for the various exposure times was 14 for the 2-h, 6 for the 0.5- and 24-h, and 2 for the 4- and 8-h groups. Bars, SEM. * Different from corresponding IC value at a 2-h exposure time (P < 0.05). ** P < 0.01.
are presented in Fig. 3B. The mean IC_{50} values for the 0.5-, 2-, 4-, 8-, and 24-h exposure times were 68.7, 28.6, 11.0, 6.34, and 2.80 µg/ml, respectively. The mean IC_{50} values were 11.4, 4.21, 3.40, 2.50, and 0.656 µg/ml, respectively. Decreasing the exposure from 2 to 0.5 h increased the IC_{50} 3-fold and prolonging the exposure from 2 to 24 h decreased the mean IC_{50} 6-fold. This indicates that exposure time is a critical determinant of MMC activity in bladder tumors, with increased activity after prolonged exposure.

Concentration-Time-Effect Relationships. The data from the multiple exposure time experiments from 6 tumors were computer fitted to the pharmacodynamic relationship C^* \times T = k (Equation C). Fig. 4 shows the plot of the IC_{50} and IC_{90} versus exposure time in a tumor. From this relationship, the pharmacodynamic parameters n, k, C_{min}, T_{min}, and (C \times T)_{min} were calculated, and the data for 6 tumors are summarized in Table 3. These data show that, on average, a 2.5-h exposure to 3 µg/ml of MMC was required to inhibit tumor LI by 50% and a 7-h exposure to 8 µg/ml of MMC for a 90% inhibition.

**DISCUSSION**

We report here the pharmacodynamics of MMC in human bladder cancer, using the native state cultures of freshly excised tumors. This type of tumor explant culture offers several advantages over existing monolayer, suspension, or soft agar methods. These advantages include maintenance of three-dimensional architecture, cell heterogeneity, cell-cell interactions, limited tumor dissociation, and a high success rate of patient tumor culture. Multicellular tumor spheroids have been used to study drug penetration and drug effects. The advantages of spheroids is the maintenance of a multicellular system. However, the spheroid system is labor intensive and costly (31). The native state culture is relatively easy because it requires only dissection of the tumor and does not require mechanical disassociation into single cells. In the present study, the anti-proliferative effect of the drug was quantitated from the extent of inhibition of tumor LI. This may be a more realistic measure of antitumor effect than colony formation which measures only those cell populations forming colonies (32).

The chemosensitivity of human bladder cancer has been studied by several other groups of investigators using established cell lines (11-16). We compared the inhibitory concentrations of MMC obtained in the present study using the native state cultures of tumors from individual patients to the literature values obtained using different methods to culture human bladder tumor cell lines and different chemosensitivity assays (Table 4). This comparison shows (a) greater variability between IC_{50} and IC_{90} values in the native state cultures than that in the cell line studies, (b) lower sensitivity to MMC in the native state cultures, as compared to the established cell lines, (c) lower drug sensitivity of a spheroid cell culture than a monolayer culture of the same cell line, and (d) that the drug sensitivity of spheroid cultures is more comparable than the monolayer or soft agar cultures to the values determined from the present study. Several factors may have contributed to the lower and more variable drug sensitivity of the primary human tumors. The selected cell lines may be inherently more sensitive. For example, 1 of the 14 patient tumors evaluated in the present study had IC_{50} values similar to those determined for the cell lines. The human explants consist of heterogeneous cell types, while continuous cell lines are relatively homogeneous. There are also differences in drug penetration, cell cycle distribution, cellular metabolism, and oxygen status between the multicellular tumor explants and the cells cultured as a monolayer. Reduced sensitivities of cells cultured as multicellular spheroids or as a bolus on collagen gel compared to monolayer cultures have been reported (33-36). This increased resistance may result from the maintenance of cell-cell interactions which are retained in the three-dimensional state. Miller and Heppner have shown that drug-treated monolayer cultures transferred and cultured as a bolus on collagen recovered more readily than when they were transferred and cultured as new monolayers (37). This behavior is attributed to the maintenance of cell-cell interactions and not the cell-collagen interactions (38).

In recently reported in vitro chemosensitivity assays, drug sensitivity of a tumor was often defined by a dichotomous outcome variable, i.e., sensitive or insensitive. This was measured as a reduction of cell viability below an arbitrary threshold after exposure to drug concentrations such as 1- or 10-fold peak plasma concentrations. For instance, sensitivity was defined as >50% reduction in tumor colonies formed in a colony-forming assay (39). A 70% reduction in viability was chosen as a criterion for sensitivity for bladder tumor aggregates cultured in suspension (40) and in solid tumors cultured using the native state technique (19). A different approach was used in the present study. No assumptions were made regarding sensitivity; rather, the IC_{50} and IC_{90} of the tumor were determined. This approach avoided the use of an arbitrary sensitivity threshold. Furthermore, the IC values, as a continuous outcome variable, could be used to establish intersubject variability in chemosen-


Table 3 Pharmacodynamic parameters for MMC activity in patient bladder tumors

<table>
<thead>
<tr>
<th>Patient</th>
<th>n</th>
<th>k</th>
<th>( R^2 )</th>
<th>( E_{50} ) (µg/ml)</th>
<th>( T_{50} ) (h)</th>
<th>( C \times T )</th>
<th>IC(_{50}^*) (µg/ml)</th>
<th>IC(_{90}^*) (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50% inhibition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 &amp; 5</td>
<td>1.20</td>
<td>4.65</td>
<td>1.00</td>
<td>2.18</td>
<td>1.83</td>
<td>1.39</td>
<td>3.99</td>
<td>2.04</td>
</tr>
<tr>
<td>7</td>
<td>1.30</td>
<td>22.4</td>
<td>0.643</td>
<td>4.34</td>
<td>3.35</td>
<td>14.5</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.54</td>
<td>27.7</td>
<td>0.998</td>
<td>4.39</td>
<td>2.86</td>
<td>12.5</td>
<td>5.78</td>
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<tr>
<td>9</td>
<td>0.771</td>
<td>6.22</td>
<td>0.994</td>
<td>2.42</td>
<td>3.14</td>
<td>7.62</td>
<td>4.13</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>1.02</td>
<td>2.67</td>
<td>0.753</td>
<td>1.64</td>
<td>1.62</td>
<td>2.65</td>
<td>0.995</td>
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<tr>
<td>14</td>
<td>1.56</td>
<td>22.2</td>
<td>0.935</td>
<td>3.99</td>
<td>2.56</td>
<td>10.2</td>
<td>6.39</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.23 ± 0.305</td>
<td>14.3 ± 11.0</td>
<td>3.16 ± 1.22</td>
<td>2.56 ± 0.702</td>
<td>8.58 ± 4.69</td>
<td>5.71 ± 4.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90% inhibition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.11</td>
<td>26.9</td>
<td>0.998</td>
<td>5.01</td>
<td>4.53</td>
<td>22.7</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.18</td>
<td>165</td>
<td>0.996</td>
<td>11.2</td>
<td>9.56</td>
<td>108</td>
<td>48.3</td>
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<tr>
<td>8</td>
<td>1.32</td>
<td>173</td>
<td>0.781</td>
<td>10.4</td>
<td>7.88</td>
<td>82.0</td>
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<td>0.948</td>
<td>51.5</td>
<td>0.998</td>
<td>7.36</td>
<td>7.77</td>
<td>57.2</td>
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<tr>
<td>11</td>
<td>1.59</td>
<td>146</td>
<td>0.966</td>
<td>8.17</td>
<td>5.12</td>
<td>41.8</td>
<td>12.5</td>
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<tr>
<td>14</td>
<td>1.35</td>
<td>106</td>
<td>0.812</td>
<td>8.27</td>
<td>6.13</td>
<td>50.6</td>
<td>37.3</td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>1.25 ± 0.222</td>
<td>111 ± 61.0</td>
<td>8.40 ± 2.21</td>
<td>6.83 ± 1.91</td>
<td>60.4 ± 30.4</td>
<td>34.4 ± 22.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Two-h MMC exposure.

Table 4 Comparison of MMC activity in primary tumor cultures to that in other culture systems

Results of MMC activity from the cultured patient bladder tumors determined in the present study were compared to values from the literature using different human bladder tumor cell lines, culture conditions and viability assays.

<table>
<thead>
<tr>
<th>Culture system</th>
<th>Cell line</th>
<th>Viability assay</th>
<th>Exposure time (h)</th>
<th>IC(_{50}^*) (µg/ml)</th>
<th>IC(_{90}^*) (µg/ml)</th>
<th>Ratio to native state</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native state</td>
<td>Primary</td>
<td>[(^{3}H)]Thd incorporation autoradiography</td>
<td>0.5</td>
<td>11.4</td>
<td>68.7</td>
<td>*</td>
</tr>
<tr>
<td>Native state</td>
<td>Primary</td>
<td>[(^{3}H)]Thd incorporation autoradiography</td>
<td>1</td>
<td>7.34^a</td>
<td>40.9^a</td>
<td>*</td>
</tr>
<tr>
<td>Native state</td>
<td>Primary</td>
<td>[(^{3}H)]Thd incorporation autoradiography</td>
<td>2</td>
<td>4.21</td>
<td>28.6</td>
<td>*</td>
</tr>
<tr>
<td>Native state</td>
<td>Primary</td>
<td>[(^{3}H)]Thd incorporation autoradiography</td>
<td>24</td>
<td>0.656</td>
<td>0.925</td>
<td>2.80</td>
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<tr>
<td>Monolayer RT-112</td>
<td>CFA^a</td>
<td></td>
<td>0.5</td>
<td>1.96</td>
<td>35(\times)11</td>
<td></td>
</tr>
<tr>
<td>Monolayer RT-112</td>
<td>CFA</td>
<td>1</td>
<td>0.92</td>
<td>44(\times)11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monolayer RT-112</td>
<td>CFA</td>
<td>2</td>
<td>0.42</td>
<td>68(\times)11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monolayer T-24</td>
<td>[(^{3}H)]Leu incorporation</td>
<td>2</td>
<td>0.10^f</td>
<td>42(\times)12</td>
<td></td>
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<tr>
<td>Monolayer KK-47</td>
<td>CFA</td>
<td>2</td>
<td>0.46</td>
<td>9(\times)151 (\times)13</td>
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<tr>
<td>Monolayer KW-103</td>
<td>CFA</td>
<td>2</td>
<td>0.22</td>
<td>159 (\times)65 (\times)13</td>
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<tr>
<td>Monolayer R-T4</td>
<td>CFA</td>
<td>2</td>
<td>0.28</td>
<td>15 (\times)45 (\times)13</td>
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<tr>
<td>Monolayer KK-47</td>
<td>CFA</td>
<td>24</td>
<td>0.023</td>
<td>29 (\times)55 (\times)13</td>
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<tr>
<td>Monolayer KW-103</td>
<td>CFA</td>
<td>24</td>
<td>0.024</td>
<td>24 (\times)64 (\times)13</td>
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<tr>
<td>Monolayer R-T4</td>
<td>CFA</td>
<td>24</td>
<td>0.059</td>
<td>33 (\times)47 (\times)13</td>
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<tr>
<td>Soft agar MGH-U1</td>
<td>CFA</td>
<td>1</td>
<td>0.48</td>
<td>15 (\times)14</td>
<td></td>
<td></td>
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<tr>
<td>Soft agar MGH-U2</td>
<td>CFA</td>
<td>1</td>
<td>1.38 (\times)10^3</td>
<td>5320(\times)14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft agar R-T4</td>
<td>CFA</td>
<td>1</td>
<td>0.0815</td>
<td>90(\times)14</td>
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<tr>
<td>Monolayer MGH-U1</td>
<td>CFA</td>
<td>1</td>
<td>0.50</td>
<td>15 (\times)44 (\times)15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spheroid MGH-U1</td>
<td>CFA</td>
<td>1</td>
<td>0.93</td>
<td>15 (\times)44 (\times)15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spheroid MGH-U1</td>
<td>CFA</td>
<td>1</td>
<td>2.03</td>
<td>7 (\times)20 (\times)15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monolayer RT-112</td>
<td>CFA</td>
<td>24</td>
<td>0.0137</td>
<td>68(\times)16</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Present study.
^a Estimated value, calculated using Equation C for comparison with data from Refs. 14 and 15.
^f CFA, colony-forming ability; BrdUrd, bromodeoxyuridine.
^f Determined graphically; value was not reported in the reference.

sitivity and relationships with other tumor properties such as grade, stage, and ploidy.

In our study, all bladder tumors were sensitive to MMC. However, the sensitivity was highly variable with a 60-fold interindividual difference in the IC\(_{50}\) and 30-fold difference in the IC\(_{90}\). The variable sensitivity was partly due to the heterogeneous nature of the patient tumors. We observed an inverse correlation between the MMC activity and the tumor proliferative activity (\(P < 0.001\)), i.e., higher MMC concentrations were needed to achieve cytotoxicity in the more rapidly proliferating tumors. There was a trend of decreasing activity of MMC in the aneuploid and high stage tumors compared to the diploid and low stage tumors. The differences between groups were not statistically significant. Studies to examine the correlation of drug sensitivity with these parameters in a larger patient population are ongoing.

The present study examined the activity of MMC in tumors cultured under oxygenated conditions. The mean diameter of the tumor specimens cultured here was 1 mm, representing a cross-section of 130 cells. Oxygen may penetrate nonvascularized tissue to depths of up to 0.25 mm (41). Thus, the cultured tumors consisted of a mixture of oxic subpopulations in the periphery and hypoxic subpopulations in the center. It has been reported that hypoxic cells are more susceptible to bioreductive alkylating agents such as MMC (42). In the cultured tumors, there was no difference in cell proliferation or drug activity for any specific region of the tumor, i.e., center or periphery. This suggests the heterogeneity in tissue oxygenation did not con-
tribute to the heterogeneity in tumor response to MMC.
Separate questions which need to be addressed are the therapeutic end point and the ability of the tumor cells to recover from the MMC effect. Treatment failure is presented as tumor recurrence. A comparison of the tumor cell recovery after treatment by MMC at different concentrations is warranted. In addition, the correlation between inhibition of tumor LI and clinical response is unknown. A prospective trial is necessary to confirm the in vitro chemosensitivity as a therapeutic end point.

The pharmacodynamic study demonstrated the relationship between the drug concentration and exposure time to the drug effect. MMC activity increased with exposure time. The mean IC₅₀ and IC₉₀ were 2- and 4-fold lower, respectively, when the exposure time was increased from 2 to 8 h. Further increasing the exposure times to 24 h reduced the IC₅₀ and IC₉₀ by 4- and 2-fold, respectively. Analysis of the data using the C₄₀ × T = k relationship showed that a Cmin of 3 μg/ml and a Tₐ₉₀ of 2.5 h were needed to produce a 50% inhibition and 8 μg/ml and 7 h for a 90% inhibition. The average value of n was 1.23. According to Equation C, an n value of 1 indicates an inverse linear relationship between the effective concentration and exposure time. This implies that an increase in exposure time will lead to a proportional decrease in drug concentration to produce the same effect. An n value of >1 indicates a slightly greater dependence on the concentration than exposure time for the overall effect. These data indicate that the therapeutic efficacy of intravesical MMC can be improved by either increasing the drug concentration at the target site or increasing the dwelling time.

To relate the in vitro tumor pharmacodynamic data to the in vivo findings, the pharmacokinetics of MMC at the target sites are needed. The target sites include the urothelium and different tissue layers of the bladder. Concurrent studies in our laboratories have examined the urine concentration-time profiles of MMC in patients given intravesical therapy and the MMC concentration-tissue depth profiles in the bladders of dogs and patients given the same intravesical dose of MMC. A comparison of the drug concentration at different tissue layers with the IC and the location of the tumors showed that, while 100% of patients with Ta tumors would have received sufficient MMC concentrations for a 90% inhibition of tumor LI, only 25% of the patients with T₁ tumors would have received sufficient MMC concentrations for 90% inhibition of the tumor LI. A detailed discussion is provided in a separate publication.

In summary, our study using the native state culture of bladder tumors from 14 patients indicates that these tumors were sensitive to MMC, irrespective of their degree of malignancy. However, the data show a large individual variability in response to MMC. The drug activity was lower in the more malignant tumors; higher concentrations were needed for tumors with a higher LI. The effect of MMC depended on concentration and exposure time, with enhanced activity at increased exposure time. Data concerning the tumor chemosensitivity demonstrated here, and the pharmacokinetics in urine and in tissue demonstrated in concurrent studies, indicate that the variable patient response rate (ranging from 10 to 50%) is due to interindividual differences in the pharmacokinetics and tumor chemosensitivity. These data further provide the pharmacological basis of the clinical observation of differential response of bladder cancers to MMC. The lower efficacy of intravesical MMC in the high stage invasive tumors as compared to the low stage superficial tumors is consistent with the lower chemosensitivity of the high stage tumors and with the lower concentration in the deeper tissue layers. Enhancement of MMC activity may be achieved by increasing the area under the drug concentration-time profile in urine and tissue by (a) reducing urine volume, (b) increasing the dwelling time, and (c) increasing drug absorption across the urothelium. The observed relationships among the IC of MMC with the tumor stage, LI, and ploidy warrant further studies to establish the value of these prognostic indicators.

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PHARMACODYNAMICS OF MMC IN BLADDER CANCER
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