Penetration of Mitomycin C in Human Bladder

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ABSTRACT

The penetration of mitomycin C (MMC) in bladder tissue was studied in patients who received intravesical chemotherapy at the time of radical cystectomy. An intravesical dose of MMC (20 mg/40 ml) was instilled and maintained in the bladder for 60 to 120 min at which time the solution was drained. Within 10 to 60 min after draining the drug solution, the bladder vasculature was ligated, and the bladder was removed. Tissues were sectioned serially in layers parallel to the urothelium and analyzed for MMC concentration. Of the 24 patients evaluated, 17 patients had a low final MMC concentration in urine (<66 µg/ml) or had the MMC solution drained more than 30 min before ligation of the blood vessels. Among these 17 patients, the concentration in the urothelium was measurable in only 4 patients, while the concentrations in deeper tissues were not measurable. In the remaining 7 patients where the urine concentration was > 120 µg/ml and where the vasculature was ligated within 30 min after the MMC solution was drained, the bladder wall contained significant MMC concentrations. The drug penetration was studied in the latter 7 patients, using sections of bladder wall that were grossly normal and non-tumor bearing. Concentrations in the bladder wall declined semilogarithmically with tissue depth from the urothelium to the deep muscle and reached a plateau at about 2000 µm depth. The median MMC concentrations were 5.6 µg/g in the urothelium and lamina propia interface, 2.7 µg/g in the lamina propia, and 0.9 µg/g in the muscularis. The distance over which the MMC concentration decreased by one-half was about 500 µm. The concentration ratio between the urine and urothelium/lamina propia interface was about 35-fold. The mean plasma concentrations were 0.003, 0.1, and 0.4% of the mean concentration in urine, urothelium, and the averaged bladder tissue concentrations, respectively. Paired superficial tumor and normal tissues were obtained from 5 bladder. In 4 of 5 cases, the concentration in tumors was higher than in normal tissues, while the reverse was seen in the remaining tumor. In one sessile bladder tumor a complete concentration-depth profile could be obtained. While the concentrations in the tumor were 2-3-fold higher than that in the adjacent normal tissue, the rate of concentration declined with respect to tissue depth and hence the distance over which the MMC concentration decreased by one-half was similar in both tumors. These data established the pharmacokinetic advantage of intravesical therapy in patients where the tumor-bearing bladder tissues receive at least 250-fold higher concentration than the systemic host tissues.

The tissue concentration-depth profiles were analyzed by two kinetic models, i.e., the distributed model which combines a drug diffusion process and a drug removal process by the perfusing blood, and the simple diffusion model which does not include drug removal by tissue blood flow. The observed logarithmic decline of drug concentration with respect to tissue depth is consistent with the distributed tissue pharmacokinetic model. The drug removal by the tissue blood flow is further supported by the low drug concentration in tissues of the 17 patients in which the MMC solution was removed 30 min before ligation of the bladder vasculatures and by the data in rabbits showing that tissue concentrations in bladders with intact blood flow declined with a half-life of 4 min after removing the drug solution. These data confirm the important role of blood flow in removing drug from the tissue. A comparison of the kinetics of MMC penetration in human bladders with the previous data in dogs (M. G. Wientjes, J. T. Dalton, R. A. Badalament, J. R. Drago, and J. L-S. Au, Cancer Res., 51: 4347-4354, 1991) shows nearly identical kinetic parameters in the two species. The kinetic parameters and the distributed model can be used to project the drug concentration at various tissue depths for a given urine concentration.

INTRODUCTION

 Transitional cell carcinoma of the bladder is a solid tumor which, because of its anatomical location, is accessible to surgical and regional treatment. Tumor recurrence is common and can be accompanied by stage and/or grade progression. Intravesical chemotherapy is used in combination with transurethral resection for treatment and prophylaxis of recurrent and/or multifocal superficial bladder cancer. Compared to patients treated by surgery alone, those receiving adjuvant intravesical chemotherapy of MMC, doxorubicin, or thiopeta have a reduced tumor recurrence rate (1-3). The target cells for intravesical chemotherapy may be present in the bladder cavity and at various tissue layers in the bladder wall. Superficial tumors appear to respond more favorably to intravesical chemotherapy than do tumors which have invaded the lamina propria and muscularis (4). Patient and animal studies in our laboratory showed that the variability in urine pharmacokinetics, drug delivery to the tumor, and tumor chemosensitivity may contribute to the varying patient responses (5-7). The ease of drug penetration in the superficial and deeper tissues and the drug concentrations at various tissue depths in the bladder wall are therefore important determinants of treatment effectiveness.

Aeikens et al. (8) have previously reported MMC concentrations in patient bladder tissue. However, data were limited and showed variable tissue concentrations in the mucosa, while the profile of drug penetration into the deeper tissues was not examined. Our earlier studies in dogs showed a steep MMC concentration decline over the urothelium, followed by a slower decline in the lamina propria and the muscularis (7). Median tissue concentrations in the muscularis of the dog bladder wall were approximately 1 µg/g, a concentration which inhibits the proliferation of about 17% of human bladder tumor histocultures (9). The present investigation was undertaken to establish the concentration of MMC in the human bladder as a function of tissue depth. A tissue pharmacokinetic model is proposed to relate the urine concentration to the tissue concentration. This study was done in patients who received intravesical therapy at the time of radical cystectomy. Additional studies to examine the effects of processing time during tissue harvesting on the tissue pharmacokinetics and the rate of equilibration between urine and tissue concentrations were performed in dogs and rabbits.

MATERIALS AND METHODS

Chemicals. Clinically formulated MMC for the patient studies was purchased from Bristol Myers Co. (Wallingford CT). MMC for the animal studies was a gift from Bristol Merck Co., and Porfimerin was a gift from the American Cyanamid Co. (Pearl River, NY). High-performance liquid chromatography analysis showed that MMC and Porfimerin were >99% pure. Agents used for anesthesia and euthanasia for the animal studies were of USP grade. Ethyl acetate and acetonitrile were purchased from Fisher Scientific Co. (Fair Lawn, NJ). All chemicals were used as received.

Surgical Procedures. Surgery on patients was performed under general anesthesia. Radical cystectomy was performed on patients with histological confirmation of primary invasive urothelial cancer or a high index of suspicion of bladder cancer in conjunction with prostatic or colorectal cancer. These...
patients had been previously treated by transurethral resection of bladder tumors. For this study, the patients received intravesical chemotherapy immediately prior to radical cystectomy. During the operation, a sterile 18 French Foley catheter was placed into the bladder, the urine was drained, and 20 mg MMC in 40 ml water were instilled into the bladder through the catheter and were maintained in the bladder for 60 to 120 min. In some patients, urine samples (0.1-ml) were taken throughout this period. After the 60-120-min instillation period, the MMC solution was drained and an aliquot was taken for drug analysis. A 10-ml blood sample was taken from a peripheral vein at the same time. The remaining bladder vasculatures were ligated and the bladder was excised. In the initial studies, the bladder vasculature was ligated more than 30 min after the drug solution was drained. In later studies, the surgical technique of cystectomy was modified as follows, so that the blood supply was interrupted within 5 min after the drug solution was drained and to avoid dilution of the drug solution by urine flow. During surgery, the ureters were ligated after bilateral pelvic lymphadenectomy. The superior vesical arteries and veins were ligated bilaterally leaving the posterior vascular pedicles to perfuse the bladder. The bladder was detached from surrounding structures and the urethra was isolated. After draining the bladder contents and transecting the urethra, and the vascular pedicles were transected using hemostatic clips and clamps. The bladder was immediately removed and opened, and within 15 min after draining the MMC solution, transmural portions of tissue without apparent lesions were obtained. When possible, transmural samples of tissues with tumor lesions were also collected. Most tumors were papillary tumors and, because of the lack of a flat surface, could not be used to study the tissue concentration-depth profiles. The papillary tumors were cut in sections to compare the drug concentrations in tumor and normal tissues. The normal tissue sections were used to establish the tissue concentration-depth profiles and the kinetic parameters of drug penetration. In one patient whose tumor had a flat surface, the concentration-depth in the tumor-bearing tissue was determined. The urachal surface area of the specimen was approximately 3 cm². The tissue was frozen on a flat stainless steel plate cooled on dry ice. Liquid nitrogen was poured over the tissue for rapid freezing. The remaining tissue was frozen on a flat stainless steel plate cooled on dry ice within 2 min after ligation of the blood supply. Liquid nitrogen was poured over the tissue for rapid freezing. The time between surgical removal of the bladder and freezing of the bladder tissue specimens was less than 4 min. Tissues, plasma, and urine samples were stored frozen at -70°C.

Effect of Lag Time between Cessation of Blood Flow and Tissue Freezing. This was studied in male or female beagle dogs (Marshall Farms, N. Rose, NY) weighing 9.03 ± 1.72 kg. The animals were fasted overnight before the experiment but were allowed free access to water. A 20-gauge angiocatheter was inserted in the cephalic vein for administration of anesthetics and a 16-gauge, 5.25-inch angiocatheter was placed in the right jugular vein for blood sampling. A urethral catheter was inserted for dose instillation. A size 14 French Foley catheter was used for female dogs, and an 8 or 10 French Foley catheter was used for male dogs. Animals were sedated with i.v. acepromazine (0.5 mg/kg) to facilitate catheterization. Anesthesia was induced by i.v. injection of sodium phenobarbital (24 mg/kg). In earlier studies we observed that urine production in dogs is variable, which results in large variations in the urine MMC concentration (7). To avoid this effect, the ureters were ligated. An anesthetized dog was placed in supine position, and the abdomen opened by midline incision. The ureters were ligated at approximately 5 cm distal to the kidney. Animals received a MMC solution (0.5 mg/ml) via the Foley catheter in a volume of 25 to 40 ml, depending on the bladder capacity titrated by hydrostatic pressure. Appropriate volume was instilled to reach a hydrostatic pressure of ~10 cm. At this pressure, the bladder is not overstretched. The dwell time of the drug solution was 120 min. Five min before removal of the bladder, the bladder contents were drained, the urethra and arterial blood supply were clamped, and the bladder was rapidly removed. Animals were euthanized with an overdose of pentobarbital. Tissue sections of approximately 2 x 2 cm surface area were cut from the dome and laterally from the left, posterior, and right sides. One section was frozen on a flat stainless steel plate cooled on dry ice within 2 min after ligation of the blood supply. Liquid nitrogen was poured over the tissue for rapid freezing. The remaining tissue sections were kept at room temperature and subsequently frozen at 5, 15, and 30 min post-tissue harvesting. Different tissue sections were subjected to different time delays in a randomized fashion.

Bladder Wall Concentrations during and after Drug Instillation. The rate of equilibration between urine and bladder wall concentrations of MMC was determined in rabbits. Rabbits were selected for this study because of their relatively low cost compared to the dog. New Zealand male rabbits, weighing 3–4 kg, were obtained from George Johnson Rabbity (Mt. Victory, OH). Anesthesia was induced with i.m. ketamine (50 mg/kg) and xylazine (8 mg/kg) and maintained with i.v. pentobarbital (12 mg/kg) given via an ear vein catheter. The bladder was catheterized with a 12 French Foley catheter via the urethra and was washed with physiological saline until the urine was clear. The abdominal cavity was entered through a midline abdominal incision and the two ureters were ligated. MMC (10 mg/10 ml) was instilled in the bladder for 5, 30, or 120 min. The bladder was excised at the end of each instillation period, as described for the dogs. A single bladder wall specimen was obtained from the right lateral side and immediately frozen. Blood and urine samples were obtained at the end of the drug instillation period. A separate experiment determined the decline of tissue concentrations with time after removal of instillate. The drug solution was drained from the bladder after 120 min and the bladder was rinsed with physiological saline. At 5, 30, or 60 min later, the bladder wall specimen was obtained and frozen.

Sample Analysis. Bladder tissue samples were analyzed as described previously (10). Briefly, the first 80 μm of the tissue, directly in contact with the instillation fluid, were trimmed off to avoid contamination by the dosing solution. The frozen tissues were cut serially into 40-μm sections parallel to the urachal surface. When necessary, multiple samples were pooled for analysis. Tissue depths were expressed as the midpoint depth of the pooled samples. Tissue sections were homogenized and extracted with ethyl acetate. Portofromycin was used as the internal standard. High pressure liquid chromatography was performed using a reversed-phase C₁₈ column (Pecosil; 83 x 4.6 mm; 3 μm particle size; Perkin Elmer, Norwalk, CT) with an aqueous mobile phase containing 2.5% acetonitrile and 5 mM phosphate buffer (pH 6.9). The flow rate was 1.5 ml/min, and UV detection was at 365 nm. Plasma and urine samples were analyzed as previously described (11).

Tissue Pharmacokinetic Model. A major objective of the present study was to establish an appropriate tissue pharmacokinetic model and to obtain the kinetic parameters of MMC penetration in human bladders. The human bladder wall can be viewed as a thin diffusion barrier (the urothelium) covering a capillary perfused tissue layer (the connective tissue and muscle layers). The urothelium in human bladders is about 200 μm thick (Fig. 1). Capillaries do not penetrate the urothelium. The surface cells of the urothelium (umbrella cells) have thickened membrane plaques at the luminal surface and are connected with tight junctions, characteristic of mammalian urothelium (12). The thickened membranes and the tight junctions contribute to the permeability barrier formed by this cell layer (12). The subepithelial connective tissue and muscles are loosely packed tissues perfused by capillaries.

The concentration decline in the capillary-perfused bladder wall was analyzed by two tissue pharmacokinetic models. The distributed model (equation 1) was first discussed by Dedrick et al. (13, 14).

\[ C_{\text{depth}} = (C_{\text{uro}} - C_{\text{b}}) \times \left( e^{-\frac{\text{depth}}{v_{\text{1/2}}}} \right) + C_{\text{b}} \]  

where \( C_{\text{depth}} \) is the concentration at the tissue depth. The \( C_{\text{b}} \) term reflects the mixed venous and arterial blood concentration. Half-width (\( v_{\text{1/2}} \)) is the thickness of the tissue over which the concentration declines by one-half. \( C_{\text{uro}} \) is the concentration at the interface between urothelium and lamina propria. The application of this model for the bladder wall concentration-depth profile has been discussed elsewhere (7). The distributed model incorporates two processes that determine the concentration-depth profile. These are (a) diffusion of drug through the tissue, which depends on the concentration gradient, and (b) removal of drug by the capillaries, a process dependent on the concentration difference between the tissue and the perfusing blood. In the bladder wall, arterial blood enters in the trigone area, perfuses the bladder wall from the base to the dome, and venous blood returns in the same tissue, collecting in venous plexi on the serosal surface of the bladder wall, and drains into the vesical veins. Venous blood leaves the bladder at the point of entry of the arteries (13). Due to the recycling of blood in the bladder wall, deep tissue concentrations equilibrate with a mixture of arterial blood containing low drug concentration and venous blood containing high drug concentrations. Hence, the concentration in the deep tissue is expected to be higher than the arterial blood concentration and lower than the venous blood concentration. A fraction of the drug proportional to \( C_{\text{depth}} \) is removed by capillary blood flow each time the drug moves past a capillary. The number of capillaries encountered by the drug increases as the distance increases. Hence \( C_{\text{depth}} \) declines exponentially with respect to tissue depth. Assuming insignificant tissue binding and that tissue concentration is in equilibrium with blood concentration, \( C_{\text{depth}} \) approaches \( C_{\text{b}} \).
The concentration-time profiles of MMC in urine were analyzed in two patients according to our previously used equations (5), with a minor modification (equations 3 and 4):

\[ C_u = \frac{Dose}{V_u} e^{-k_0 \cdot t} \]  

(3)

and

\[ V_u = V_a + V_{res} \]  

(4)

where \( V_u \) is the volume of the urine at time \( t \); \( V_a \) is the volume of the MMC dosing solution; \( V_{res} \) is the volume of residual urine present in the bladder at the time of instillation; \( C_u \) is the urine concentration; \((k_0 + k_3)\) is a hybridized first order rate constant describing drug absorption, degradation, metabolism, and tissue binding. Our previous model included a urine production term (5). This term was deleted for the cystectomy patients in the present study because there was no significant drainage into the bladder due to early ligation of the ureters.

The average tissue concentration reflects the total tissue exposure to MMC. The rate of equilibration between urine and tissue concentration was determined. The half-life \((t_{1/2})\) of reaching equilibrium was estimated by equation 5.

\[ \frac{C_{mean}}{C_u} = F_{eq} \times (e^{-0.935 \times r^*} - e^{-0.935 \times 1}) \]  

(5)

where \( C_u \) is the urine concentration at the time of drainage, \( F_{eq} \) is the fractional bladder wall concentration when equilibrium has been reached, and \( r^* \) is the time after drainage of the bladder. \( r^* \) equals 0 during instillation.

Statistical analysis was by analysis of variance (SAS Institute). Differences between groups were determined using the paired or unpaired two-tailed Student’s \( t \) tests and the Wilcoxon rank sum test at a 5% level of significance. Correlations were evaluated with the Pearson’s correlation coefficient, and frequencies were evaluated with Fisher’s Exact test.

**RESULTS**

**Patient Characteristics.** We evaluated the bladders of 24 patients who underwent radical cystectomy for muscle-invading tumors. In seven patients, drug concentration in superficial and deep tissues and concentration-depth profiles were obtained. In four patients, the concentrations in the superficial tissues including tumor and normal tissues, but not the deep tissues, were measurable. In 13 patients, tissue

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**References**

[16] and [17].
Pathological tumor stage was based on the tumor, nodes, and metastasis staging system (20, 21). NA, not applicable.

Bladder cancers (5). The average residual urine volume at the time of early ligation of ureters, drainage of the bladder and interruption of bladder function were measured in patients with superficial bladder cancer (5). Plasma concentrations were <1 µg/g, and in 13 cases the highest bladder wall concentrations were near or below the detection limit of 0.1 µg/g. In 17 patients, MMC concentrations were obtained and the peak tissue concentrations were >2 µg/g (Table 2). The Cb values were considerably more variable than Curo and Cmean.

Concentrations were <1 µg/g and the peak tissue concentrations were >2 µg/g (Table 2). The Cb values were considerably more variable than Curo and Cmean.

Concentration-depth profiles observed in our previous study in patients with superficial bladder cancer (5), probably because the procedures used in surgery, e.g., suction, was more effective in emptying the bladder than was the drainage procedure used in nonsurgical patients. The combined drug absorption and degradation rate constant were 0.012 and 0.003 min⁻¹ for the two patients, which were similar to the previous results (5).

In 17 patients including the 4 patients who showed detectable concentrations in the superficial tissues, the highest bladder wall concentrations were <1 µg/g, and in 13 cases the highest bladder wall concentrations were near or below the detection limit of 0.1 µg/g. In these patients, the MMC solution was removed >30 min before ligation of blood vessels or had low concentration (<66 µg/ml). Tissue concentration-depth profiles could not be determined in these patients. To achieve higher urine and tissue concentrations, several procedures were implemented during the course of the study, i.e., early ligation of ureters, drainage of the bladder and interruption of blood perfusion as late as possible in the procedure, and freezing of the bladder specimen in the operating room rather than after transport to the pathology area. These procedures resulted in substantially increased drug concentrations in urine and bladder tissue.

**Tissue Concentration-Depth Profiles.** In 7 patients, MMC concentration in the urine recovered at 120 min ranged from 120 to 620 µg/ml and the peak tissue concentrations were >2 µg/g (Table 2). Concentration-depth profiles were obtained in these patients. There was a correlation between Cb and Curo (r² = 0.85; P < 0.005). The Cu was 20-50-fold higher than Curo, with mean and median ratios of about 35 to 1. Drug concentration further declined as the drug penetrated the lamina propria and the muscle layers and reached a relatively constant level at a depth of approximately 2000 to 4000 µm. The Cu values were considerably more variable than Curo and Cmean. The ratio of Cu to Cb was about 1860 to 1. Fig. 2 shows the tissue concentration-depth profiles. Both mean and median values are shown to illustrate the interpatient variability. The median tissue concentration were one-half the mean concentrations due to the fact that the concentrations in three patients were substantially higher than in the other four patients. Computer-fitting the data with the distributed model (equation 1) and the diffusion model (equation 2) showed that the observed data were in better agreement with the values predicted by the distributed model (r² = 0.94 ± 0.07) than by the diffusion model (r² = 0.65 ± 0.24). Analysis by Akaike information criterion confirmed that in all seven cases, the distributed model gave a significantly better fit. The wv of the concentration decline in the capillary-perfused tissue was about 500 µm.

**Plasma Concentrations.** Plasma concentrations were obtained in four of the seven patients with measurable tissue concentrations. Plasma concentrations were in the order of one million-fold lower than urine concentrations and 10-1000-fold lower than the average tissue concentrations (Table 2). In our previous studies on patients undergoing intravesical chemotherapy, plasma concentrations ranged from 2.1 to 181 ng/ml for treatments given within 3 days after transurethral resection of superficial bladder cancer (5). Plasma concentrations in the radical cystectomy patients (95 ± 80 ng/ml) were higher than in the superficial bladder cancer patients (43 ± 59 ng/ml; Ref. 5). However, this difference was not statistically significant by unpaired t test or Wilcoxon rank sum test.

Effect of Time Delay before Tissue Removal and Freezing. We reported that the time interval between clamping the blood supply to the bladder and freezing the bladder tissue is critical to obtaining accurate tissue concentration-depth profiles (10). Delays of greater than 1 h resulted in substantial changes in the profiles, i.e., a lower urothelial concentration and a larger wv, reflecting a more shallow concentration decline in the tissue (10). In radical cystectomy patients, a time delay of approximately 10-20 min between ligation of the major perfusing blood vessels to the bladder and freezing of the bladder wall tissue was unavoidable due to the complexity of the surgical procedure. Modification of the surgical techniques as described in "Materials and Methods" decreased the delay time between ligation of the blood supply and tissue freezing from 30-90 to 10-20 min. The effect of a 2-30-min delay on the bladder tissue concentration-depth was determined in dogs. Our previous studies (7, 10) indicate a 2-5-fold variability in MMC penetration into different parts of the dog bladder, probably related to the heterogeneity in the tissue composition and blood supply (7). With this large variability, it was deemed unlikely that the effect of a time delay on tissue concentration profiles could be determined in individual animals. The experimental design was therefore to examine the time delay effect using the mean data from 4 animals. From each animal, tissues from different anatomical sites, i.e., dome, left, posterior, and right side of the bladder, were taken and subjected to different time delays. The time delays between cessation of blood flow and tissue freezing were randomized with respect to the tissue sites; e.g., the dome and the left side of one animal had delays of 2 and 30 min, while the same tissues of another animal had delays of 30 and 2 min. The randomization was to avoid

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**Table 1 Patient characteristics**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>Tumor stage a</th>
<th>Tumor grade a</th>
<th>Time TURBT a to cystectomy (days)</th>
<th>Remarks</th>
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<td>A</td>
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<td>M</td>
<td>P1N0M0</td>
<td>II</td>
<td>14</td>
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</tr>
<tr>
<td>B</td>
<td>58</td>
<td>M</td>
<td>P4N1M0, colon cancer</td>
<td>III</td>
<td>93</td>
<td></td>
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<tr>
<td>C</td>
<td>56</td>
<td>M</td>
<td>P3N1M0</td>
<td>II</td>
<td>25</td>
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<tr>
<td>D</td>
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<td>F</td>
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<td>III</td>
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<td></td>
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<tr>
<td>E</td>
<td>75</td>
<td>M</td>
<td>P3N0M0</td>
<td>III</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>61</td>
<td>M</td>
<td>P1N0M0</td>
<td>III</td>
<td>7</td>
<td>Urothelial dysplasia, prior irradiation, profuse hemorrhagic radiation cystitis</td>
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<td>G</td>
<td>78</td>
<td>M</td>
<td>P3N0M0, bladder cancer</td>
<td>P1N0M0, prostate cancer</td>
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<tr>
<td>H</td>
<td>57</td>
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<tr>
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<td>M</td>
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<td>III</td>
<td>6</td>
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<tr>
<td>J</td>
<td>54</td>
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<td>K</td>
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<td>M</td>
<td>P1N0M0 bladder cancer</td>
<td>P1N0M0, prostate cancer</td>
<td>48</td>
<td>Gleason score 7</td>
</tr>
</tbody>
</table>

**Notes:**
- Tumors were graded with a modified Armed Forces Institute of Pathology protocol; grade 1, well differentiated; grade 2, moderately differentiated; grade 3, poorly differentiated.
- Pathological tumor stage was based on the tumor, nodes, and metastasis staging system (20, 21).
- NA, not applicable.
- Transurethral resection of bladder tumor.

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* Transurethral resection of bladder tumor.

**Tumor downstaged by 4500 cGy preoperative irradiation.**
Table 2 Concentrations of MMC in bladder tissue, urine, and plasma after intravesical instillation in patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>C_{uro} (μg/ml)</th>
<th>C_{mean} (μg/ml)</th>
<th>C_{700} (μg/ml)</th>
<th>W_{1/2} (μm)</th>
<th>C_{u} (μg/ml)</th>
<th>C_{b} (μg/ml)</th>
<th>Ratio ( \frac{C_{uro}}{C_{b}} )</th>
<th>Ratio ( \frac{C_{uro}}{C_{mean}} )</th>
<th>Ratio ( \frac{C_{uro}}{C_{p}} )</th>
<th>Ratio ( \frac{C_{mean}}{C_{b}} )</th>
<th>Ratio ( \frac{C_{u}}{C_{b}} )</th>
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<tbody>
<tr>
<td>D</td>
<td>27.8 ± 9.3</td>
<td>14.4 ± 10.1</td>
<td>3.2 ± 6.2</td>
<td>495 ± 82</td>
<td>622 ± 110</td>
<td>1.1 ± 0.8</td>
<td>0.08 ± 0.04</td>
<td>0.03 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>F</td>
<td>4.3 ± 1.2</td>
<td>2.1 ± 0.6</td>
<td>0.3 ± 0.1</td>
<td>478 ± 120</td>
<td>120 ± 4</td>
<td>1.1 ± 0.1</td>
<td>0.1 ± 0.04</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>G</td>
<td>14.18 ± 2.8</td>
<td>1.84 ± 0.4</td>
<td>0.49 ± 0.04</td>
<td>150 ± 526</td>
<td>0.48 ± 0.5</td>
<td>0.4 ± 0.1</td>
<td>0.09 ± 0.04</td>
<td>0.01 ± 0.01</td>
<td>0.01 ± 0.01</td>
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<tr>
<td>H</td>
<td>22.24 ± 9.2</td>
<td>13.46 ± 3.9</td>
<td>3.98 ± 7.3</td>
<td>661 ± 168</td>
<td>0.73 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.09 ± 0.04</td>
<td>0.01 ± 0.01</td>
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</tr>
<tr>
<td>I</td>
<td>4.6 ± 2.3</td>
<td>2.95 ± 1.5</td>
<td>1.49 ± 0.2</td>
<td>525 ± 168</td>
<td>1.17 ± 0.1</td>
<td>1.1 ± 0.1</td>
<td>0.09 ± 0.04</td>
<td>0.01 ± 0.01</td>
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</tr>
<tr>
<td>J</td>
<td>2.7 ± 1.4</td>
<td>1.98 ± 0.9</td>
<td>0.91 ± 0.4</td>
<td>957 ± 132</td>
<td>0.23 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>0.01 ± 0.01</td>
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<tr>
<td>K</td>
<td>5.56 ± 1.5</td>
<td>2.66 ± 0.4</td>
<td>0.42 ± 0.2</td>
<td>468 ± 260</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.01 ± 0.01</td>
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<tr>
<td>Mean</td>
<td>11.65 ± 3.91</td>
<td>5.62 ± 1.56</td>
<td>1.56 ± 0.53</td>
<td>533 ± 331</td>
<td>0.54 ± 0.09</td>
<td>0.5 ± 0.1</td>
<td>0.06 ± 0.04</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
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</tr>
<tr>
<td>SD</td>
<td>10.01 ± 3.68</td>
<td>5.69 ± 1.47</td>
<td>1.47 ± 0.24</td>
<td>242 ± 206</td>
<td>0.48 ± 0.08</td>
<td>0.4 ± 0.1</td>
<td>0.08 ± 0.04</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.02 ± 0.01</td>
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</tr>
<tr>
<td>Median</td>
<td>5.56 ± 2.28</td>
<td>2.66 ± 0.91</td>
<td>0.91 ± 0.45</td>
<td>495 ± 280</td>
<td>0.48 ± 0.09</td>
<td>0.48 ± 0.1</td>
<td>0.09 ± 0.04</td>
<td>0.01 ± 0.01</td>
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</table>

\( C_{uro} \), \( C_{700} \), and \( C_{2000} \) are the concentrations at 200 μm (urothelial basement membrane where tumors are located), 700 μm (lamina propria, \( T_1 \) tumors), and 2000 μm (muscularis, \( T_2 \) tumors). \( C_{mean} \) is the mean concentration between 80 and 3000 μm.

\( \alpha \) ND, not determined.

Figure 2. Bladder wall concentration versus depth profiles. Bladders were removed from patients treated with intravesical MMC. A through-cut specimen of tissue (non-tumor-bearing) was obtained and snap-frozen. Symbols and bars are mean + 1 SD and median concentrations (n = 7). Lines connect the experimental data points and are not model-fitted lines. A representative profile is given in the inset, where the experimental data (dots) were computer-fitted to the distributed model.

Fig. 3. Bladder wall concentrations as a function of duration of drug instillation. Rabbits received intravesical instillation of MMC (10 mg/10 ml) for 5, 30, or 120 min, after which the drug solution was drained and the bladder was immediately removed. In other groups given a 120 min instillation, the bladder was removed at 5 or 30 min after the drug solution was drained. Data were computer-fitted using equation 5. Symbols and bars are mean + 1 SD values for 3–5 animals. Line, model-predicted values. Concentrations at 30- and 60-min postinstillation were not detectable.

The mean bladder wall concentrations, expressed as fractions of urine concentrations, are shown in Fig. 3. The tissue to urine concentration ratios after instillations of 5 and 30 min were similar, indicating that the equilibrium was established within several minutes. In several animals (with intact blood flow) where the instillate was removed at 120 min and the bladder was rinsed with saline, the bladder wall concentrations declined rapidly during the first 5 min. MMC was not detectable in the bladder wall after 30 min. This supports the rapid drug removal by the perfusing blood. The mean tissue concentrations were computer-fitted with equation 5 for a monoeponential increase to an equilibrium level, followed by a monoeponential decline after removal of the instillate. The \( t_{eq} \) of equilibration was determined to be 3.6 min, indicating a rapid equilibrium between the urine and tissue concentrations.

**MMC Concentrations in Tumor and Adjacent Normal Tissues.**

MMC concentrations in tumor and grossly normal tissues in the same bladders were compared in 5 patients. The most commonly found form of transitional cell carcinoma is a papillary tumor consisting of stalks of tumor tissue growing from a central area and lacking a smooth surface. For these tumors, the concentration-depth profiles could not be determined with accuracy, and only the average concentration in the tumor tissue could be determined. In 4 patients, the
MMC concentration in the superficial papillary tumors was higher than the adjacent apparently normal tissue, while the reverse was observed for the fifth patient (Table 3). The difference was not statistically significant ($P = 0.21$, Fisher's Exact test). One patient had a smooth, sessile transitional cell carcinoma. We were able to determine the concentration-depth profiles in the tumor and the adjacent normal tissue. The tumor tissue had higher concentrations than the normal tissue (Fig. 4). However, in light of the slope of the decrease in concentration, the $w_{ts}$ were similar.

**DISCUSSION**

Data on drug penetration to tumor-bearing organs are important to determine whether effective drug concentrations are achieved at the tumor site. The bladder tissue penetration data have most significance for superficial bladder cancer patients who often receive intravesical therapy. The present study, because of the tissue requirements, could not be done in patients with superficial disease and was performed in patients with muscle-invading disease. Two potential differences between the two groups of patients with superficial or invasive diseases with respect to the conditions of the bladder wall and the vasculature, in part due to the nature of the surgical procedures, are as follows: (a) Cessation of bladder blood flow by surgical intervention affects the drug removal by the capillaries. The effect of a time delay between blood vessel ligation and freezing of tissue samples was studied in dogs. The results showed a 60% overestimation of the $w_{ts}$ and small changes in the tissue concentrations for a 15-min time delay. These changes are relatively minor compared to the interpatient variability in these parameters. (b) Physical manipulation of the bladder during surgery might have affected the urothelial integrity and drug absorption. Likewise, more extensive lesions in the cystectomy patients than the superficial cancer patients might have altered the drug penetration characteristics. However, the plasma data, which reflect the extent of systemic absorption, showed no difference in the MMC concentrations in the cystectomy and superficial bladder cancer patients (5), suggesting that the effect of bladder manipulation on drug absorption, if any, was minimal. Hence, while these potential differences are recognized, the existing data suggest that the tissue pharmacokinetic data obtained in the cystectomy patients are applicable to the superficial bladder cancer patients.

The major conclusions of the present study are two-fold. First, the present study is one of several designed to identify the causes of the variable and incomplete response of superficial bladder cancer to intravesical chemotherapy. Previous studies investigated the urine pharmacokinetics in patients, the MMC concentrations required for a cytotoxic effect on histocultures of patient bladder transitional cell carcinoma, and the penetration of MMC in dog bladders (5–7). Based on the data of the previous studies and on the assumption that the penetration of MMC in dog bladders is similar to that in human bladders, we presented computer simulated data to show that the clinical observation of a lower efficacy of intravesical MMC therapy in high stage tumors as compared to the superficial low stage tumors is due in part to the ineffective drug concentration in the deep tissues and to the higher drug concentration needed to inhibit the high stage tumor (7). Data of the present study showed nearly identical values of the $C_{tu}/C_{u}$ ratio and the $w_{ts}$ in human and dog bladders. This verifies the previous assumption that the dog is a good model for studying MMC penetration in humans and supports the proposed pharmacological basis of the varying and incomplete patient response to intravesical chemotherapy.

Secondly, the distributed tissue pharmacokinetic model, which combines a drug diffusion process and a drug removal process by tissue blood flow, was found appropriate to describe tissue MMC concentration-depth profiles. The drug removal by the tissue blood flow is further supported by the low drug concentration in tissues of the 17 patients in whom the MMC solution was removed 30 min before ligating the bladder vasculatures and by the data in rabbits showing a rapid equilibration between the urine and tissue concentrations and a rapid tissue concentration decline in the presence of intact blood flow. The data further established the kinetic parameters of MMC penetration in the human bladder, including: (a) $C_a$ to $C_{u}$ ratio (35 to 1); (b) $C_{tu}$ to $C_t$ ratio (1860 to 1); (c) $C_{u}$/ratio (839 to 1); (d) $C_{mean}/C_p$ ratio (246 to 1); (e) $w_{ts}$ (500 μm); (f) plateau tissue concentration at 2000 to 4000 μm depth; and (g) rate of equilibrium between the urine and tissue concentrations ($t_{eq}$ of 4 min). Intravesical chemotherapy is used to treat superficial tumors, i.e., $T_a$ and $T_t$ tumors located in the urethelium and $T_I$ tumors in the lamina propria. The large ratio of tissue to plasma concentrations confirms the pharmacokinetic advantage of intravesical therapy to give high drug concentration in tumor-bearing bladder tissue and a minimal systemic drug exposure. The rapid equilibration between urine and tissue concentrations suggests that the tissue exposure to MMC is determined by the instillation period and the urine concentrations, the two parameters which can be readily controlled during a treatment.

In the present study, the tissue pharmacokinetic data were determined using primarily bladder sections with no apparent lesions. In one case where paired tumor and normal tissues were obtained and compared, the tumor-bearing tissue showed higher concentrations but a similar $w_{ts}$. Further studies using tumor-bearing tissues are needed to confirm the tumor tissue pharmacokinetics.

The establishment of the tissue pharmacokinetic model and the numerical values of the kinetic parameters permits the projection of tissue concentration-depth profiles based on the $C_{tu}$. Urine samples and $C_{tu}$ can be readily obtained while tissue concentrations, in part due to the limited human tissue availability, cannot be routinely obtained. $C_{tu}$ depends on several environmental factors, including the dose, volumes of the dosing solution and urine and, because of the pH-
dependent stability of MMC, the urine pH (5). In a separate study, we used computer simulations to project different Cu values by altering these environmental factors and to use the projected Cu values to generate the tissue concentration-depth profiles (19). The tissue concentrations were compared to the concentrations needed to inhibit the proliferation of human bladder tumors (6, 9) to estimate the treatment efficacy. Based on the simulated tissue concentrations and the resulting treatment efficacy, an optimized treatment regimen was selected (19) and is currently being evaluated in a phase III trial.

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Penetration of Mitomycin C in Human Bladder

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