Bladder Tissue Uptake of Mitomycin C during Intravesical Therapy Is Linear with Drug Concentration in Urine

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ABSTRACT

The design of an ongoing Phase III study of intravesical mitomycin C therapy to treat bladder cancer is partly based on the assumption that drug penetration into bladder tissue is linearly related to drug concentration. The present study was designed to (a) test this assumption and (b) to compare drug concentrations in tumor and adjacent normal tissues in human bladders. We previously reported the uptake kinetics of a 20-mg dose in dog and human bladders (M. G. Wientjes et al., Cancer Res., 51: 4347–4354, 1991, and Cancer Res., 53: 3314–3320, 1993). The present study used a 40 mg/20 ml dose. Serial blood and urine samples were taken from dogs during the 120-min instillation. Bladder tissues were harvested from dogs and patients at the end of instillation. A comparison of the results of the present and previous studies indicates identical tissue penetration kinetic parameters in dogs for the two doses, i.e., a ~30-fold concentration drop across the urothelium and a half-width of ~500 μm. In addition, the average tissue concentration in dog and human bladders attained with the 40-mg dose (8.77 μg/g in dogs and 7.55 μg/g in humans) was about twice that achieved with the 20-mg dose (4.33 μg/g in dogs and 3.91 μg/g in humans). In dogs, the plasma concentration of MMC reached a steady state within 10 mm; the mean maximal plasma concentration was 8.5 ng/ml. This plasma concentration to tissue concentration, and a pharmacodynamic assumption used in the design of the ongoing Phase III clinical trial.

INTRODUCTION

Transitional cell carcinoma of the bladder, because of its anatomical location, is accessible to surgical and regional treatment. Tumor recurrence after surgical removal is common and is often accompanied by stage and/or grade progression. Adjuvant intravesical chemotherapy is used for treatment and prophylaxis of recurrent and/or multifocal superficial bladder cancer. Compared to patients treated by surgery alone, patients receiving adjuvant MMC3 therapy have a reduced tumor recurrence rate (1, 2). The target cells for intravesical chemotherapy may be present in the bladder cavity and in various tissue layers of the bladder wall. Our previous studies have established the MMC concentration in human or dog bladder as a function of tissue depth (3–5). These studies show that variabilities in urine pharmacokinetics, drug delivery to tumor cells, and tumor chemosensitivity contribute to the varying patient responses. A tissue pharmacokinetic model was developed to relate urine concentration to tissue concentration, and a pharmacodynamic model was developed to correlate drug exposure with antitumor effect. The model predicted that a treatment regimen using a higher dose (40 mg) and treatment conditions that minimize dilution/reduction of drug concentration in urine (i.e., minimizing residual urine volume by ultrasound-guided emptying of the bladder, reducing urine production by dehydration, using the smallest possible dosing solution volume, and minimizing drug degradation by urine alkalinization) would increase the response rate by 22% compared to the standard regimen (i.e., 20 mg/20 ml, uncontrolled residual urine volume, uncontrolled hydration status, and uncontrolled urine pH; Refs. 6 and 7). These data have led to an international Phase III efficacy trial to compare the optimized and standard regimens. One of the assumptions used in the model is that MMC uptake in bladder tissue is linearly related to its concentration in urine. The present study was undertaken to test this assumption in dogs and humans. The dog study provided information on the bladder penetration kinetics, whereas the human study provided information on average tissue concentrations. The study further compared the drug penetration in normal and tumor bladder tissues in humans.

MATERIALS AND METHODS

Chemicals. MMC was a gift from Bristol-Myers Squibb Co. (Wallingford, CT), and porfiromycin was from American Cyanamid Co. (Pearl River, NY). HPLC analysis showed that MMC and porfiromycin were >99% pure. Ethyl acetate and.

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3 The abbreviations used are: MMC, mitomycin C; HPLC, high-performance liquid chromatography.
Mitomycin C Penetration in Bladder Tissue

Acetaminophen were purchased from Fisher Scientific Co. (Fair Lawn, NJ), and desipramine hydrochloride was purchased from Sigma Chemical Co. (St. Louis, MO). All chemicals were used as received. Agents used for anesthesia and euthanasia were of USP grade.

In Vivo Study in Dogs. Male or female beagle dogs (HRP, Inc., Kalamazoo, MI) weighing 9.1 ± 1.2 kg (mean ± SD) were used. The animals were fasted overnight before the experiment but were allowed free access to water until the experiment. One angiocatheter (20 gauge, 4 inch) was inserted in the cephalic vein for administration of anesthetics, and another (16 gauge, 5.25 inch) was placed in the right jugular vein for blood sampling. French Foley catheters (size 13 or 14 for female dogs, and size 8 or 10 for male dogs) were inserted for dose instillation and sampling of the bladder contents. Animals received a sedative, acepromazine (0.5 mg/kg), by i.v. injection to facilitate catherization.

Animals were given an intravesical dose of MMC (40 mg in 20 ml of saline) for 2 h. All experiments were started between 1 and 3 p.m. Animals were anesthetized for the duration of the experiment. After emptying urine from the bladder, MMC was instilled via the Foley catheter. The catheter was then flushed with air to ensure complete delivery of the intravesical dose. Serial blood and urine samples were collected before and during the instillation period and immediately before surgical removal of the bladder. To obtain urine samples, a 10-ml syringe was connected to the outlet of the Foley catheter. Five ml of the bladder contents were withdrawn to fill the catheter with urine. The latex catheter was then pierced with a 1-ml syringe (30-G needle), and samples (0.1 ml each) were taken. The contents of the catheter were then returned to the bladder, followed by a volume of air equal to the catheter volume so that the urine in the catheter was replaced by air. By this method, the collection of each urine sample was completed within 2 min.

At the end of the 2-h treatment period, the bladder was exposed by a midline incision, and the dome and left and right lateral sides of the bladder were marked with superficial stitches of surgical silk. To maintain the concentration gradient between urine and tissue and to avoid "washout" of the drug during the sample processing, it was necessary to maintain the drug solution in the bladder until just prior to clamping the blood supply. After draining the bladder contents at 120 min, the urethral arterial blood supply was clamped, and the bladder was rapidly removed. Tissue sections of approximately 2 × 2-cm surface area were cut from the dome and left and right lateral sides. The sections were rapidly frozen on a flat, stainless steel plate cooled on dry ice. Liquid nitrogen was poured over the tissue to ensure the rapid freezing. The procedures between removing the intravesical dose and freezing the bladder tissue specimens required less than 5 min. A short time interval between clamping the blood supply to the bladder and freezing the bladder tissue is critical for obtaining accurate tissue concentration profiles (5). Animals were euthanized with an overdose of i.v. pentobarbital immediately after removal of the bladder.

Degradation of MMC in Bladder Tissues. To determine whether drug degradation accounted for drug loss, drug stability in bladder tissues and PBS was examined. Bladder tissues taken from three dogs were cut into small pieces (about 3 × 3 mm) and incubated with 10 ml of MMC solution (5 μg/ml, pH 7.4, in 0.01 M potassium monophosphate) at 37°C for 2 h in a Petri dish. The MMC solution was then removed, and the Petri dish was sealed by stopcock grease (Dow Corning Co., Midland, MI) to avoid dehydration and was stored under room temperature (18°C). Three to four tissue pieces weighing about 0.04 g in total were removed at time 0 and daily for 7 days. Separate samples with only MMC solution (10 ml) and without tissues were prepared; 100-μl aliquots were obtained at the same time as tissue sampling. The MMC concentrations at various times were compared to the concentrations at time 0 to determine the extent of drug degradation.

In Vivo Study in Patients. Surgery on patients was performed under general anesthesia. Radical cystectomy was performed on patients with histological confirmation of primary invasive urothelial cancer or with a high index of suspicion of bladder cancer in conjunction with prostatic or colorectal cancer. All patients had been previously treated by transurethral bladder tumor resection. For this study, 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 a solution of MMC (40 mg/20 ml water) was instilled into the bladder and maintained in the bladder for 2 h. Afterward, the MMC solution was drained. 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 the surrounding structures and the urethra was isolated. After draining the bladder contents and transecting the urethra, the vascular pedicles were transected using hemostatic clips and clamps. The bladder was immediately removed and opened. Within 15 min after draining the MMC solution, transmural
The concentration-time profiles of MMC in the bladder content during intravesical therapy were analyzed using Eqs. B and C to obtain the values of the residual urine volume ($V_{res}$), urine production rate ($k_d$), and absorption/degradation rate constant ($k_a + k_d$). The area under the concentration curve ($AUC_{bladder}$) was calculated using the linear trapezoidal rule. The fraction of the dose recovered was calculated as amount recovered via the Foley catheter divided by administered dose.

### Table 1 Pharmacokinetics of MMC in dogs

<table>
<thead>
<tr>
<th>$k_0$ (ml · min⁻¹)</th>
<th>$V_{res}$ (ml)</th>
<th>$k_a + k_d$ (min⁻¹)</th>
<th>$AUC_{bladder}$ (mg · min/ml)</th>
<th>Final urine concentration ($C_{ub}$, mg/ml)</th>
<th>Final plasma concentration (ng/ml)</th>
<th>pH</th>
<th>% of dose recovered at 2 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.094</td>
<td>6.13</td>
<td>0.0024</td>
<td>136</td>
<td>0.92</td>
<td>5.1</td>
<td>6.9</td>
</tr>
<tr>
<td>SD</td>
<td>0.056</td>
<td>3.77</td>
<td>0.0020</td>
<td>22</td>
<td>0.24</td>
<td>2.3</td>
<td>0.4</td>
</tr>
</tbody>
</table>

### Analysis of MMC Tissue Concentration-Depth Profiles Using the Distributed Model

We have shown that drug penetration in bladder is described by the distributed model, which considers drug removal by capillary drainage in addition to drug diffusion (11). The decline of drug concentrations with respect to the penetrated tissue depth is described by the following equation:

$$C_i = (C_0 - C_b) e^{-\frac{0.693}{w_{1/2}}} + C_b,$$

where

$$w_{1/2} = 0.693 \frac{[pa + q]}{D}$$

### Analysis of Concentration-Time Profiles of MMC in Urine

The concentration-time profiles of MMC in urine during the 120-min instillation period were analyzed using Eqs. B and C.

$$C_u = \frac{Dose}{V_u} e^{-k_a t + k_d t}$$

$$V_u = V_0 + k_d t + V_{res}$$

$V_u$ is the volume of urine at time $t$; $V_0$ is the volume of MMC dosing solution (20 ml); $k_d$ is the zero-order rate constant describing urine production; $V_{res}$ is the theoretical volume of residual urine present in bladder at time of instillation; $k_a$ is the first-order rate constant describing drug absorption into the systemic circulation; and $k_d$ is a hybridized first-order rate constant describing degradation, metabolism, and tissue binding of MMC. In this study, systemic blood concentrations of MMC were at least 10,000-fold less than the urine concentration. Hence, the transfer of MMC from systemic circulation to blad-
Mitomycin C Penetration in Bladder Tissue

**RESULTS**

**Degradation of MMC.** Upon incubation at room temperature, MMC concentration decreased slightly each day. The total concentration decline after 7 days was $4.6 \pm 1.1\%$ in the absence of dog bladder tissues and $6.3 \pm 1.2\%$ in the presence of tissues. The small difference in the two rates indicates minimal enzymatic degradation of MMC in bladder tissue.

**Urine and Plasma Pharmacokinetics in Dogs.** Fig. 1 shows the average urine concentration-time profiles in dogs. The decline of urine concentrations over time could be due to urine production, absorption, degradation, and metabolism. The pH of the instillate ranged from 6.6 to 7.5 in these animals. We and others (8, 12) have shown that MMC is relatively stable within this pH range, with a degradation half-life of 38 h. Hence, nonenzymatic degradation in urine was not a significant factor in this study. The values of the urine pharmacokinetic parameters, $(k_r + k_d)$, $k_0$, and $V_{res}$, are shown in Table 1. About 90% of the dose was recovered at the end of the 2-h experimental period, indicating that <10% dose was in the bladder tissue and systemic circulation.

Plasma concentrations of MMC reached a plateau value within 10–15 min, which was maintained throughout the instillation period (Fig. 1). The maximal plasma concentrations ranged from 2.2 to 8.5 ng/ml (mean, 5.1 mg/ml) and were 80,000-fold lower than the urine concentrations at corresponding times. These plasma concentrations are comparable to the level obtained at the 20-mg dose (i.e., 1.8–30 ng/ml; mean, 8 ng/ml; Ref. 5), indicating that the higher 40-mg dose did not yield a significantly greater systemic exposure.

**Tissue Concentration-Depth Profiles in Dogs.** Fig. 2 shows the mean MMC concentration-depth profile in 15 tissues (3 tissues per animal) and a representative profile in a single tissue. The decline of tissue concentrations with respect to tissue depth away from the urothelium is described by the distributed anatomical sites of bladder (i.e., right and left lateral sides and dome) show an average variation of about 10%, indicating no site dependence in bladder tissue penetration kinetics. This is consistent with our previous findings in dog and human bladders (5, 13).

MMC Concentrations in Human Bladder Tumor and Adjacent Normal Tissues. MMC concentrations in tumor and grossly normal tissues in the same human bladders were compared. Drug concentration in tumor tissues was significantly higher (i.e., urinary excretion) was considered negligible and was not included in the model. $AUC_{\text{bladder}}$ (area under the drug concentration-time profiles in urine) was calculated by the linear trapezoidal rule. The fraction of the dose recovered was calculated as the amount recovered in urine drained through the Foley catheter divided by the amount administered.

The analysis of tissue concentration-depth profiles by Eq. A and the analysis of urine concentration-time profiles by Eqs. B and C were performed using the nonlinear least-squares estimation of SAS/STAT software (SAS Institute Inc., Cary, North Carolina). The analysis provided the parameters $C_0$, $C_b$, $w_{12}$, $V_{res}$, $k_0$, and $(k_r + k_d)$.

### Table 2

Concentration of MMC in human bladder tissues after instillation of 40 mg/20 ml

<table>
<thead>
<tr>
<th>Bladder</th>
<th>Normal (µg/g)</th>
<th>Tumor (µg/g)</th>
<th>Ratio of tumor:normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.54</td>
<td>14.3</td>
<td>1.50</td>
</tr>
<tr>
<td>2</td>
<td>5.94</td>
<td>7.35</td>
<td>1.24</td>
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<tr>
<td>3</td>
<td>2.53</td>
<td>4.22</td>
<td>1.69</td>
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<tr>
<td>4</td>
<td>8.22</td>
<td>9.95</td>
<td>1.21</td>
</tr>
<tr>
<td>5</td>
<td>11.2</td>
<td>14.2</td>
<td>1.21</td>
</tr>
<tr>
<td>Mean</td>
<td>7.48 ± 3.36</td>
<td>9.98 ± 4.35</td>
<td>1.38 ± 0.21</td>
</tr>
<tr>
<td>SD</td>
<td>2.28</td>
<td>2.95</td>
<td>0.20 ± 0.12</td>
</tr>
<tr>
<td>$P$</td>
<td></td>
<td></td>
<td>0.014$^{a}$</td>
</tr>
</tbody>
</table>

$^{a}$ NA, not applicable.

$^{a}$ Significantly different from normal tissue concentrations.

$^{a}$ Significantly different from 1.
higher than the concentration in the adjacent normal tissue (Table 3).

Comparison of Drug Uptake in Bladder Tissues for the 20- and 40-mg Doses. Table 2 compares the results of the present study using the 40-mg dose and the results of the previous studies using the 20-mg dose (5, 13). The comparison indicates identical kinetic parameters in dog bladders, i.e., a ~30-fold concentration drop across the urothelium, and a half-width of ~500 μm. The average tissue concentration achieved with the 40-mg dose in dog and human bladders was about twice that achieved with the 20-mg dose, indicating that the tissue uptake of MMC is linearly related to its concentration in urine.

DISCUSSION

The first goal of the present study was to determine whether the bladder tissue uptake of MMC is proportional with drug concentration in urine. Because we are specifically interested in the concentration range used in the ongoing Phase III trial, we used the two doses, i.e., 20 and 40 mg per 20 ml, that are being studied in patients. The results indicate that the bladder tissue uptake of MMC is linearly related to the drug concentration in urine, which supports the assumption used in the design of the trial. Interestingly, the higher dose, although producing a higher tissue concentration, did not produce a significantly higher plasma concentration in dogs when compared to the lower dose. This is in part due to the high variability in the systemic absorption between animals. The systemic drug concentration in patients receiving the two doses are being compared in the ongoing Phase III study. The preliminary data indicate that the plasma concentrations achieved at these two doses are 20–40-fold (14) below the threshold toxic concentration of 400 ng/ml (15).

The second goal of the study was to compare the drug concentration in tumor and normal tissues. We previously reported a trend of a higher concentration in tumor tissue compared to normal tissue, although the differences were not statistically significant (13). Data of the present study confirm the previous finding and establish the significantly higher drug concentration in tumor tissues compared to the adjacent apparently normal tissues. It should be noted that the approximately 40% higher concentration in the papillary tumor might have resulted from the larger surface area available for drug absorption due to its protruding structure, as compared to the smaller flat surface of the normal urothelium.

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Bladder tissue uptake of mitomycin C during intravesical therapy is linear with drug concentration in urine.

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