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# Use of pharmacologic data and computer simulations to design an efficacy trial of intravesical mitomycin C therapy for superficial bladder cancer

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**Summary.** Treatment of superficial bladder cancers by intravesical mitomycin C (MMC) chemotherapy gives a varying and incomplete response. Our recent pharmacokinetics and pharmacodynamics studies have shown that treatment effectiveness is limited by drug degradation in acidic urine and by drug dilution due to residual urine volume and urine production. A model was developed to predict drug exposure in tumors in the bladder wall and to correlate drug exposure with antitumor effect. The model is based on the known pharmacokinetic data in patients treated with intravesical chemotherapy, drug-penetration data in the bladder wall of patients undergoing radical cystectomy, and pharmacodynamic data on patients' bladder-tumor chemosensitivity. Computer simulations based on the model were generated. The simulations predicted that changes in treatment parameters would affect the therapeutic outcome in the following rank order: dose > residual volume > urine production > dosing volume > urine pH > dwell time. Tissue exposure could be enhanced by increased dose, complete bladder emptying, reduced fluid intake, use of the minimal dosing volume, and alkalinization of the urine to a neutral pH. Increasing the dwell time from 2 to 4 h gave an insignificant improvement and posed a compliance problem. The selected optimized regimen of a 40-mg dose, no residual volume, 0.62-ml/min urine production, a 20-ml dosing volume, and alkaline urine pH yielded a calculated 8.5-fold increase in tissue exposure over that achieved by the standard regimen, which consisted of a 20-mg dose, 32-ml residual volume, 1.5-ml/min urine production, a 20-ml dosing volume, and acidic urine pH. On the basis of previously established pharmacodynamic data, we hypothesize that the increase in tissue exposure in the optimized treatment would result in a 20% improvement over the standard therapy along with an increase in the recurrence-free rate from 56% to 76% of patients. A phase III efficacy trial comparing the optimized and standard regimens is proposed.

## Introduction

The American Cancer Society estimates a yearly incidence of 50,000 new cases of bladder carcinoma in the United States [2]. Approximately 70%–80% of patients present with superficial papillary tumors and/or carcinoma in situ. Although transurethral resection is the primary treatment, 40%–80% of patients treated only by transurethral resection will develop tumor recurrence. Recurrent tumors may result from the growth of occult unresected tumor, the implantation of tumor cells into denuded surfaces during resection, or de novo tumor formation from premalignant urothelial cells. Among the patients with recurrent tumors, 10%–20% will have grade and/or stage progression [19, 22, 33]. The rationale behind the use of intravesical therapy is that it may partially or completely eliminate the existing tumor and thereby reduce the risk of tumor recurrence and progression. A literature survey has shown that adjuvant intravesical mitomycin C (MMC) may decrease the incidence of recurrence within 1 year by 2%–43% as compared with surgery alone [14].

Direct administration of chemotherapy into the bladder gives high exposure at the bladder tumor sites while sparing the patient from systemic toxicities. The current approach in intravesical therapy is to instill the drug using an empirically based dosage regimen and dwell time. Al-

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*Abbreviations:* MMC, mitomycin C; SWOG, Southwest Oncology Group; $C_u$, urine concentration; $V_u$, urine volume; $V_0$, dosing volume; $k_0$, urine production rate; $V_{res}$, residual volume; $k_a$, absorption rate constant; $k_d$, degradation rate constant; AUC, area under the concentration-time curve; $w_{1/2}$, half-width; $C^n \times T$, drug exposure; LI, labeling index; $T_{inst}$, instillation time

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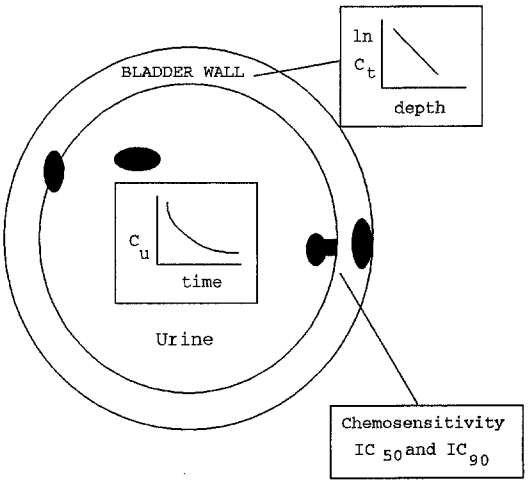


**Fig. 1.** Schematic representation of the determinants of MMC efficacy against tumor cells (*shaded symbols*) located in the bladder cavity, superficially in the urothelium (e.g., $T_a$, $T_{is}$, and $T_1$ tumors), and in deeper tissues ($T_2$, $T_3$, and $T_4$ tumors). Drug penetration into tumor cells is determined by the time-dependent changes in urine concentration (urine pharmacokinetics). The tissue concentration is further determined by the distance the drug travels in the tissue. The chemosensitivity of the tumor cells is denoted by the drug concentration needed to inhibit tumor cell proliferation by 50% and 90%, i.e., $IC_{50}$ and $IC_{90}$

though the empirical regimens have produced clinical responses, the highly variable and incomplete response rate suggest that improvement is possible. Our recent studies on MMC have addressed questions important for optimal therapy. The data show that the variable patient response to intravesical MMC may be due to the large inter- and intrasubject variability in the pharmacokinetics at the tumor sites and to the variable sensitivity of patients' tumors to MMC [6, 23, 27–30]. On the basis of these data, we hypothesize that the tumor exposure to MMC and, hence, the therapeutic efficacy of intravesical therapy can be improved substantially by modification of several treatment parameters, including the dose, dosing volume, patients' hydration status, urine pH, and dwell time. In the present report, mathematical models are proposed to describe the relationships between environmental factors (e.g., urine pH and volume), drug concentration, exposure time, and drug activity. Computer simulations were performed to rank-order the parameters that determine the tumor cell exposure to MMC, to identify the optimal conditions for these parameters, and to estimate the improvement in treatment efficacy due to optimization.

## Patients and methods

### *Model development*

*Computer simulations.* The simulations were done using an IBM-compatible, Intel 486 microprocessor-equipped personal computer (Gateway, N. Sioux City, S.D.). Software for statistical analysis (NPAR1WAY and TTEST procedures) was supplied by SAS (Cary, N.C.). Simulations used numerical integration, over 5-min discrete time intervals, programmed in SAS basic language. Selection of sample size for prospective trials was calculated using the stratified log-rank test and the chi-square test [4, 5]. The goodness of fit of different mathematical models to experimental data was compared with the Akaike Information Criterion [31].

*General approaches for simulation.* The goal of adjuvant intravesical MMC is the elimination of neoplastic cells not removed by surgery. Residual neoplastic cells are located in the urine, superficially on the lumenal bladder surface, and in the bladder wall. Figure 1 is a schematic representation of the factors that determine the therapeutic efficacy of an intravesical treatment. These factors are the pharmacokinetics in urine, the pharmacokinetics in bladder tissues, and the tumor's sensitivity to MMC. Previous pharmacodynamics studies have shown a correlation between MMC exposure and the response of human bladder tumors [23]. Hence, an increase in drug exposure is expected to yield an improved response. The tumor cell exposure to MMC is determined by several environmental and physiologic factors that can be modified as shown below. Simulations were done to depict the effect of altering these factors on the tumor cell exposure to MMC and the resulting treatment efficacy.

*Urine pharmacokinetics.* In intravesical therapy, after the bladder has been emptied by Foley catheterization, the drug solution is instilled and remains in the bladder during the 2-h treatment. Afterward, the drug solution is drained through the Foley catheter. The urine concentration, $C_u$, during drug instillation is described by the following equation:

$$C_u = \frac{\text{Dose}}{V_u} e^{-(k_a + k_d)t}, \quad (1)$$

where $V_u = V_0 + k_0 \times t + V_{res}$. Urine is continuously produced during the instillation. $V_u$ is the urine volume at time $t$; $V_o$ is the initial volume of the instillate; $k_o$ is the urine-production rate constant; $V_{res}$ is the volume of residual urine present in the bladder at the time of instillation; $k_a$ is the apparent absorption rate constant from the bladder, i.e., across the bladder epithelium and muscular layers to the capillaries; and $k_d$ is the apparent rate constant of degradation, metabolism, and/or binding to macromolecules.

*Patients.* We studied the plasma and urine pharmacokinetics of MMC in 10 patients receiving 27 treatments by a commonly used regimen of intravesical MMC (20 mg in 40 ml sterile water) [6] and determined the values for $V_0$, $V_{res}$, $k_0$, and $(k_a+k_d)$. Patient bladders, after being emptied by catheterization, still contained a large volume of urine [6]. This $V_{res}$ caused a significant dilution of the MMC concentration in urine. Similarly, intra- and interpatient variability in $k_o$ and $(k_a+k_d)$ caused significant variation in urine concentration. Bladder exposure to MMC inversely correlated with $V_{res}$, $k_o$, and $(k_a+k_d)$ [6]. In the simulations, we altered the values for these parameters to examine their relative influence on the tissue exposure.

*Control of the urine production rate and urine pH.* The control of the urine production rate and urine pH was studied in healthy volunteers. In six men (age range, from 21 to 39 years), fluid and caffeine intake was stopped at midnight and the bladder was voided at 8 a.m. Completeness of voiding was ascertained by sonography. The urine volume at the next voiding at noon was used to determine the urine production rate.

Control of the urine pH in a range from 7 to 7.5 by sodium bicarbonate treatment has been reported in several studies using doses of up to 1 g/h [1, 16, 32]. Urine alkalinization was evaluated in eight healthy volunteers (seven men aged 21–39 years and one woman aged 44 years). The baseline urine pH in individuals was determined in urine samples voided in the morning (between 10 a.m. and noon) of day 1. Sodium bicarbonate was given orally (650 mg at night and 650–1,300 mg in the next morning). The pH of urine voided in the morning on days 2 and 3 was determined. Titration of urine pH by phosphate buffer was determined using the acidic urine obtained from one subject.

*Bladder-tissue pharmacokinetics.* The following pharmacokinetic equations were established to relate the urine concentration to the concentration in tissues. Detailed discussions of the theoretical basis for these equations have been given elsewhere [29]. The absorption of a drug from

the bladder cavity into the systemic circulation involves primarily two processes of drug penetration, i.e., across the urothelium, which is not perfused by blood, and across the deeper tissues, which are perfused by the capillary blood flow. The drug transfer from the bladder cavity across the 7- to 10-cell-layer-thick urothelium, which is the diffusion barrier, is described by Fick's first law. The drug transfer across the capillary-perfused bladder wall is described by the distributed model, first discussed by Dedrick and co-workers [8, 10–12].

Equation 2 describes the concentration-depth profile in the urothelium (0–200 μm), and Eq. 3 describes the concentrations in the capillary-perfused tissue (200–4,000+ μm):

$$0\text{–}200\ \mu m: \quad C_{depth} = C_u - \frac{C_u - C_{200}}{200} \times depth \tag{2}$$

$$200\text{–}4{,}000^{+}\ \mu m: \quad C_{depth} = (C_{200} - C_b) \times e^{-\frac{0.693}{w_{1/2}} \times (depth - 200)} + C_b, \tag{3}$$

where $C_u$ is the concentration of unionized drug in the bladder cavity, $C_{200}$ is the concentration at the interface between the urothelium and the deep tissues, and $w_{1/2}$ (half-width) is the thickness of tissue over which the drug concentration declines by 50%. Within the urothelium, the decline in $C_{depth}$ is linear with depth and depends on the drug partition and diffusion (which are dependent on the ionization and lipophilicity of the drug), the concentration gradient across the urothelium, and $V_u$. At depths beyond the urothelium, the $C_{depth}$ declines exponentially from the concentration at the urothelial surface ($C_{200}$) to the averaged free blood concentration ($C_b$). A fraction of the drug proportional to ($C_{depth} - C_b$) is removed by the capillary blood flow each time the drug moves past a capillary. The number of capillaries encountered by the drug increase as the distance increases. Hence, the concentration declines exponentially with respect to tissue depth. At a depth much greater than the $w_{1/2}$, $C_{depth}$ approaches $C_b$. Note that the tissue concentration-depth profile can be generated with the knowledge of $C_u$, $w_{1/2}$, the ratio of $C_u$ to $C_{200}$, and the ratio of $C_u$ to $C_b$. $C_u$ varies for individual patients and changes with time; $w_{1/2}$ and the $C_u$:$C_{200}$ and $C_u$:$C_b$ ratios are determined by the physicochemical property of the drug and the amount of drug removed by the blood flow. These are constant parameters for a given drug in a given system and can be determined experimentally.

We studied the kinetics of penetration of MMC in the bladder wall of 21 patients who received 20 mg/40 ml of intravesical MMC 1–2 h before undergoing radical cystectomy as well as in dogs and rabbits [27–30]. Concentrations in the bladder wall were not measurable if the drug-containing urine had been removed more than 30 min before ligation of the major blood vessels, consistent with a process of rapid clearance of MMC by the bladder blood flow. In seven patients in whom bladder-wall concentrations could be evaluated, the $C_u$:$C_{200}$ ratio was about 36. The MMC concentration declined logarithmically with the distance traveled and the $w_{1/2}$ of the MMC concentration decline was about 500 μm. At a tissue depth greater than 4,000 μm, a concentration plateau was reached at a concentration of 0.10% of the $C_u$. MMC concentrations appeared to be higher in tumor tissue as compared with grossly normal adjacent tissues in five patients from whom both tissues could be obtained. The bladder-wall penetration data in patients was supported by findings obtained in dogs and rabbits [28, 30]. The animal studies further showed that (a) concentrations in the bladder wall reached steady-state equilibrium with the concentrations in the bladder contents within minutes (in the simulations, instantaneous equilibration between urine and tissue concentrations was assumed) and (b) the MMC concentration in the vesical vein, a major vessel draining the bladder, was similar to the average of the concentrations in bladder tissues, which suggests a mixing of blood in capillaries throughout the bladder prior to its entry into the systemic circulation. The experimentally determined values for $w_{1/2}$, $C_u$, and the $C_u$:$C_{200}$ and $C_u$:$C_b$ ratios were used in the simulations.

*Pharmacodynamics studies using human tumor explants.* The relationship between drug concentration, exposure time, and drug-induced inhibition of tumor cell proliferation was studied using patients' bladder tumor explants [23]. The data showed that all of the 30 human tumors responded to MMC. There was a 120-fold interpatient variability. For individual tumors, the growth-inhibitory concentration (IC) of MMC was inversely related to the exposure time.

Because the urine concentrations and, thus, the tissue concentrations do not remain constant with time, the initial drug concentration could not be used as a measure of drug exposure. A measure of total exposure was developed. We have shown that the antitumor effect of MMC is related to its concentration (C) and to the exposure time (T) according to the relationship $C^n \times T = k$ [23]; $k$ is the drug-exposure constant and has a constant value for equivalent exposures, i.e., exposures resulting in an equivalent drug effect. For example, the concentration needed to produce a 50% inhibition of tumor cell proliferation ($IC_{50}$) after a 2-h exposure will be substantially higher than the $IC_{50}$ for a 24-h exposure. However, the $C^n \times T$ product remains constant and will produce a 50% inhibition. A necessary assumption for this relationship is that any drug concentration will produce an effect, i.e., there is no activity threshold. This assumption is substantiated by the observed inhibition of tumor cell labeling index by MMC over a 50-fold concentration range [23]. Note that when n equals 1, the equation becomes $C \times T = k$. This implies that the concentration and the exposure time are equally important in determining the effect. When n is larger than 1, the concentration is more important than the exposure time, and vice versa. To generalize $C^n \times T$ from a situation in which drug concentrations remain constant with time (e.g., under culture conditions) to a situation whereby concentrations change with time (e.g., dilution by urine and degradation), $C^n \times T$ is calculated as the time integral of $C^n$ over the instillation period, $T_{inst}$ (Eq. 4). The drug exposures corresponding to 50% or 90% inhibition are denoted as $C^n \times T_{50}$ and $C^n \times T_{90}$, respectively.

$$C^n \times T = \int_0^{T_{inst}} C^n \times d(time) \tag{4}$$

Previous studies in six patients' tumors show an average n value of 1.24, with five tumors showing an n value greater than 1 and one tumor having a value lower than 1 [23]. Further statistical analysis using the Akaike Information Criterion [31] showed that the pharmacodynamics in three tumors was best described by an n value different from 1, whereas that in the other three tumors was best described by an n value equal to 1. Simulations were done using n values of 1 and 1.24.

*Simulation models.* To achieve curative therapy, we hypothesize that the MMC exposure at the tumor sites should exceed the $C^n \times T_{90}$. To obtain eradication of tumor cells, several logs of cell reduction will be needed. In a regimen of six treatments this may be achieved with a 90% reduction per treatment, corresponding to a $C^n \times T_{90}$ exposure. Tumor cells located in urine or on top of the bladder urothelium are directly in contact with the MMC dosing solution, which is manyfold higher than the $IC_{90}$. These cells are likely to be killed by the treatment. Tumor cells located in the bladder wall receive lower drug concentrations and are less likely to be killed. $T_a$ and $T_{is}$ tumors are located in the urothelium; $T_1$ tumors, in the lamina propria; and $T_2$ and $T_3$ tumors, in the corresponding depths of the muscularis layers. The drug concentration declines during penetration into the deeper tissues. Hence, the different stage tumors will be exposed to different drug concentration-time profiles. These profiles were obtained by simulations.

The simulations of tumor cell exposure to MMC under different treatment conditions used a fourstep process. (1) A treatment was selected by choosing values for each of the following parameters: drug dose and dosing volume, urine production rate, residual urine volume, urine pH, and dwell time. (2) The urine concentration-time profile was calculated from these parameters using Eq. 1. All treatment parameters except pH could be directly entered in the equation. We previously found that the $k_d$ of MMC in urine at pH 5 is 0.004 $min^{-1}$, whereas the $k_d$ at pH >7 is negligible [6]. The effect of pH was reflected by a change in $k_d$. (3) The concentration versus tissue-depth profile in the bladder wall was calculated by Eqs. 2 and 3 for each time point using the $C_u$ at that time and the established constant parameters. (4) For each depth, the concentration-time profile was converted to $C^n \times T$ using Eq. 4. By this process, a tissue concentration-time versus tissue-depth profile was generated for a selected condition. The $C^n \times T$ at the tumor site was compared with the $C^n \times T_{50}$ and $C^n \times T_{90}$ against human bladder tumors to evaluate the frequency of successful treatments. The $C^n \times T_{50}$ and $C^n \times T_{90}$ were derived from the $IC_{50}$ and $IC_{90}$, respectively, of human bladder-tumor

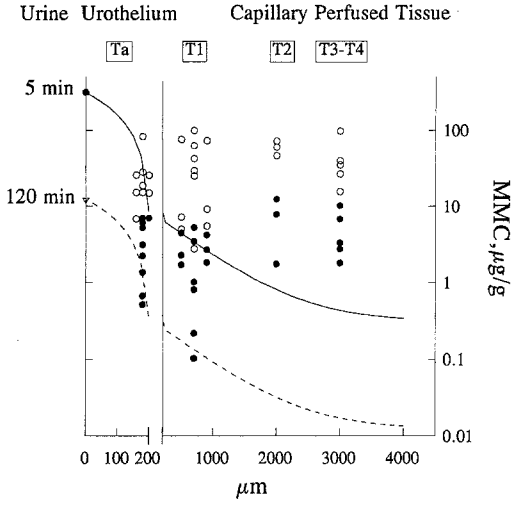


**Fig. 2.** Simulated bladder-wall tissue concentration-depth profiles obtained after a standard, uncontrolled MMC therapy (20 mg/40 ml water). These profiles were generated using Eqs. 2 and 3 and the necessary parameters ($w_{1/2}$, ratio of $C_u$: $C_{200}$ and $C_u$) were obtained from previously published studies. The tissue concentration-depth profiles obtained at 5 min (*solid line*) and 120 min (*dotted line*) after instillation of the drug into the bladder are shown and compared with the $IC_{50}$ (*filled circles*) and $IC_{90}$ (*open circles*) in 30 patients' tumors

cell proliferation after a 2-h exposure. Data on 30 tumors, including those of the previously reported 14 patients [23], were used.

*Calculation of drug efficacy.* Several prospective studies have compared surgery plus MMC therapy with surgery alone [15, 17, 21, 25]. The overall treatment effect represents a combination of the effect of MMC and that of surgery. We estimated the percentage of cure (defined as 12 months without recurrence) due to MMC treatment by Eq. 5, using the series of Niijima et al. [21] as an example. In this series, surgery alone gave a 54% (75/139) rate of cure. In the MMC arm, the overall response was 67% (93/139). MMC was effective in curing 18 (93 minus 75) of the 64 (139 minus 75) patients not cured by surgery. The MMC effect was calculated to be 28% (18/64).

$$\text{MMC effect} = \frac{F_{MMC} - F_{surgery}}{1 - F_{surgery}} \times 100\%, \quad (5)$$

where $F_{MMC}$ is the fraction of patients cured after surgery plus MMC therapy, and $F_{surgery}$ is the fraction of patients cured by surgery alone.

## Results

### *Drug concentration at the target site and pharmacodynamic relationship: the pharmacologic basis of the variable and incomplete response to intravesical MMC*

Figure 2 shows simulations of bladder-wall concentration-depth profiles after a standard, uncontrolled therapy of MMC at 20 mg/40 ml. The urine pharmacokinetic data and the tissue-penetration data used in the simulations were obtained from our previous studies in patients receiving a 20-mg MMC dose in 40 ml. The simulated tissue pharmacokinetic data were compared with the IC values in individual patients' tumors categorized by their tumor stage, i.e., $T_a$ tumors in the urothelium (depth, 0–200 μm); $T_1$ tumors in the lamina propria (200–1,200 μm); and $T_2$, $T_3$, and $T_4$ tumors in the muscle layers (1,200–4,000+ μm). The comparison suggests that tumors in the deeper bladder wall, i.e., the more invasive tumors, do not receive sufficient drug concentration. Inhibitory concentrations were achieved in the urothelium ($T_a$ tumors) but were only partially attained in the lamina propria ($T_1$), and the concentrations in the muscle layer were <20% of the $IC_{90}$ for $T_2$ tumors. These data further demonstrate the importance of urine pharmacokinetics for the treatment efficacy in $T_1$ tumors. Our previous study showed a several-fold drop in urine concentrations from the beginning to the end of treatment. The drop in the urine concentration over 120 min led to a proportional drop in tissue concentrations. Of the $T_1$ tumors, 10 of 12 (83%) received concentrations $\geq IC_{50}$ at 5 min, but only 1 (8%) received an effective $IC_{50}$ at 120 min.

To evaluate the tissue exposure over the entire instillation period, we converted the concentration-depth profiles to $C^n \times T$ versus depth profiles. A comparison of the $C^n \times T$-depth profiles with the $C^n \times T_{50}$ and $C^n \times T_{90}$ values shows that after a standard treatment, 89% (8/9) of $T_a$ tumors and 33% (4/12) of $T_1$ tumors were exposed to $C^n \times T_{50}$, whereas 22% (2/9) of $T_a$ tumors and none of the (0/12) $T_1$ tumors were exposed to $C^n \times T_{90}$ (Fig. 3). In summary, these data suggest that the variable responses in superficial tumors and the lower drug efficacy in invasive tumors are due to (a) variable concentrations at the tumor site, (b) inadequate drug concentrations in the deep tissues, and (c) lower sensitivity of the invasive tumors to MMC. The model-predicted lower sensitivity of the invasive tumors is consistent with the clinical experience [14, 19, 22, 24, 26, 33].

### *Simulations of urine and bladder-tissue pharmacokinetics and treatment efficacy under different treatment conditions*

Tumor exposure to MMC is determined by the dose, the dosing volume, the dilution of the dosing solution by the postcatheterization residual urine volume and the urine produced during treatment, the nonenzymatic drug degradation, and the exposure time. In the standard regimen, the bladder is emptied by catheterization without checking for the completeness of bladder emptying, patients are not refrained from fluid and caffeine intake, and the urine pH and urine production rate are not controlled. Under these conditions, the residual urine volume was 32.4 ± 40.3 ml (range, 0–130.7 ml), the urine production rate was 1.48 ± 1.11 ml/min (range, 0.08–4.61 ml/min), and the urine pH ranged from 5 to 8 [6].

In the simulations, these parameters were altered. The dose and dosing volume were selected, and the values for urine production rate and urine pH were based on the results obtained in healthy volunteers. The MMC doses currently used in clinical practice range from 20 mg dissolved in 20–40 ml water to 40 mg. A dose of 60 mg MMC has been used on a limited basis and requires 30 ml water for dissolution [3]. There are no conclusive clinical data to indicate the most effective dose and volume. The simu-

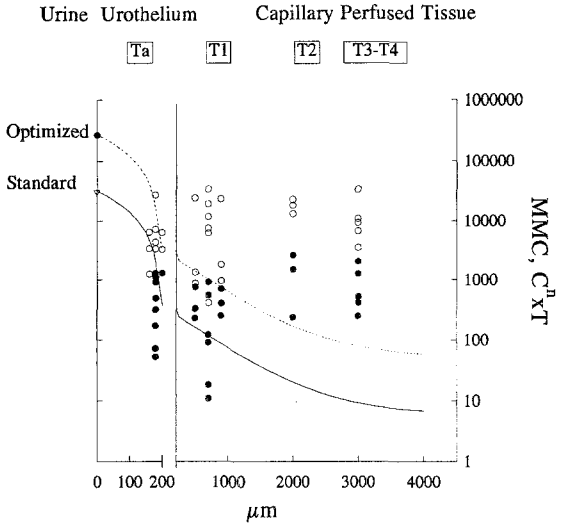


**Fig. 3.** Simulations comparing the standard, uncontrolled therapy (*dotted line*) with the optimized therapy (*solid line*). $C^n \times T_{50}$ (*filled circles*) and $C^n \times T_{90}$ (*open circles*) values were calculated from the $IC_{50}$ and $IC_{90}$, respectively, using Eq. 4 and an n value of 1.24

lations compared three doses, i.e., 20 mg/20 ml, 40 mg/20 ml, and 60 mg/30 ml. By controlling the catheter position and patient positioning and by sonographically checking the residual volume, a minimal residual urine volume of 0–10 ml can be achieved.

*Control of the urine production rate and urine pH*

Results obtained from healthy volunteers showed that the urine output, under the condition of refraining from fluid and caffeine intake overnight, was $0.63 \pm 0.20$ ml/min (range, 0.31–0.75 ml/min). For six of eight subjects, 650 mg bicarbonate given at night and repeated in the morning was sufficient to control the urine pH to above 6.5 for the next 2 h. Two subjects with acidic urine required 1.3 g bicarbonate in the morning. An alternative method to control the urine pH within a narrow range is to use buffered drug solution. In all, 60 ml 100 m*M* phosphate buffer (pH 7) was required to neutralize 50 ml acidic (pH 5.2) urine. The large volume ruled out the use of buffer.

*Simulation results*

In the simulations, each parameter was altered, independently and simultaneously, to project the target-site exposure and treatment outcome. Simulations using values of 1.24 and 1 for n in the $C^n \times T$ relationship gave qualitatively similar results. Figure 3 shows the simulations obtained with an n value of 1.24, and Table 1 summarizes the results obtained with both n values.

In Table 1, the improvement factor is the ratio of the $C^n \times T$ at 200 μm projected for the depicted altered condition to the $C^n \times T$ projected for the standard, uncontrolled regimen. The standard regimen, consisting of 20 mg MMC in 20 ml water for a 2-h instillation, is identical to the conditions used in the recently completed Southwest On-

**Table 1.** Simulations of bladder-wall exposure and treatment effect

| Variable changed | $C^n \times T$ urine | $C^n \times T$ at 200 μm | Improvement factor | Percentage of tumors exposed to $C^n \times T_{50}$, $C^n \times T_{90}$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | $T_a$ | | $T_1$ | | $T_2$ | | $T_3$–$T_4$ | |
| | | | | $C^n \times T_{50}$ | $C^n \times T_{90}$ | $C^n \times T_{50}$ | $C^n \times T_{90}$ | $C^n \times T_{50}$ | $C^n \times T_{90}$ | $C^n \times T_{50}$ | $C^n \times T_{90}$ |
| n = 1.24 | | | | | | | | | | | |
| Standard (dose = 20, $V_0$ = 20, $k_0$ = 1.48, $V_{res}$ = 32.4, pH = 5, $T_{inst}$ = 120) | 32,179 | 374 | 1.00 | 88.9 | 22.2 | 33.3 | 0 | 0 | 0 | 0 | 0 |
| Instillation vol. 20 → 40 ml | 24,779 | 288 | 0.77 | 88.9 | 22.2 | 25.0 | 0 | 0 | 0 | 0 | 0 |
| Dose 20 → 40 mg | 76,005 | 882 | 2.36 | 88.9 | 55.6 | 50.0 | 0 | 0 | 0 | 0 | 0 |
| Urine production 1.48 → 0.626 ml/min | 44,702 | 519 | 1.39 | 88.9 | 44.4 | 41.7 | 0 | 0 | 0 | 0 | 0 |
| Residual volume 32.4 → 0 ml | 62,092 | 721 | 1.93 | 88.9 | 55.6 | 50.0 | 0 | 0 | 0 | 0 | 0 |
| pH 5 → 7 | 32,237 | 432 | 1.16 | 88.9 | 22.2 | 33.3 | 0 | 0 | 0 | 0 | 0 |
| Instillation time 120 → 240 min | 32,962 | 383 | 1.02 | 88.9 | 22.2 | 33.3 | 0 | 0 | 0 | 0 | 0 |
| Optimized (dose = 40, $V_0$ = 20, $k_0$ = 0.63, $V_{res}$ = 0, pH = 7, $T_{inst}$ = 120) | 274,919 | 3,191 | 8.53 | 100 | 77.8 | 100 | 25.0 | 0 | 0 | 0 | 0 |
| High-dose combination (dose = 60, $V_0$ = 30, $k_0$ = 0.63, $V_{res}$ = 0, pH = 7, $T_{inst}$ = 120) | 332,968 | 3,865 | 10.33 | 100 | 77.8 | 100 | 25.0 | 0 | 0 | 0 | 0 |
| n = 1 | | | | | | | | | | | |
| Standard (dose = 20, $V_0$ = 20, $k_0$ = 1.48, $V_{res}$ = 32.4, pH = 5, $T_{inst}$ = 120) | 9,928 | 273 | 1.00 | 88.9 | 22.2 | 25.0 | 0 | 0 | 0 | 0 | 0 |
| Optimized (dose = 40, $V_0$ = 20, $k_0$ = 0.63, $V_{res}$ = 0, pH = 7, $T_{inst}$ = 120) | 56,871 | 1,564 | 5.73 | 100 | 77.8 | 100 | 16.7 | 0 | 0 | 0 | 0 |

The variables were altered and the resulting $C_u$ and $C^n \times T$ values were calculated using Eq. 1–4. Simulations were done using two n values, 1.24 and 1

cology Group (SWOG) trial comparing MMC and bacillus Calmette-Guérin. Optimization of all treatment parameters [dose, 40 mg; dosing solution volume, 20 ml; urine-production rate constant, 0.63 ml/min; postcatheterization urine volume, 0; pH, 7; instillation time ($T_{inst}$), 240 min] increased exposure by 6.0 (n = 1) or 8.8 times (n = 1.24). As single parameters, the rank order was dose > residual volume > urine production > dosing volume > urine pH > dwell time. For example, at n = 1.24, the increase in the dose from 20 to 40 mg gave the greatest improvement factor of 236%, whereas the increase in $T_{inst}$ from 120 to 240 min gave the smallest improvement of <5%. The reason for the insignificant effect of increased $T_{inst}$ was that urine concentrations at 120 min were <10% of the initial concentrations, which led to a minimal increase in $C^n \times T$ by an increase in dwell time beyond 120 min. The increase in exposure for the optimized regimen with a $T_{inst}$ of 120 min was 5.7-fold (n = 1) or 8.5-fold (n = 1.24). Simulation of a further increase in the dose from 40 mg/20 ml to 60 mg/30 ml gave a relatively small enhancement of 21%.

Table 1 also shows the fraction of target sites exposed to the $C^n \times T_{50}$ or $C^n \times T_{90}$. The superficial tumors, i.e., $T_a$, $T_{is}$, and $T_1$, are candidates for intravesical treatment. The expected outcome of the proposed treatment efficacy against these tumors was calculated as follows. Cure is defined as 12 months of recurrence-free survival. Recurrence at >12 months is considered to represent a new tumor. A survey of literature data on $T_a$ and $T_1$ tumors shows for surgery alone a cure rate of about 50%, with lower cure rates being found in some referral settings [14, 15, 17, 19, 21, 25]. Table 1 shows that the standard MMC treatment delivered the $C^n \times T_{90}$ to 22% of the patients with $T_a$ tumors. Hence, the standard MMC treatment would give an additional 11% cure in the 50% of the patients not cured by surgery (22% × 50%), for a total cure rate of 61% (50%+11%) by surgery plus MMC. As can be seen from Table 1, $T_1$ patients would not receive the $C^n \times T_{90}$, and the total fraction cured would remain 50% as in surgery alone. In a patient group that is equally distributed in $T_a$ and $T_1$ tumors, the total result of the standard regimen would be a 56% [0.5 × (50%+61%)] cure. These calculated responses are comparable with those reported in several clinical trials of $T_a$ and $T_1$ tumors [15, 17, 21, 25]. This agreement supports the hypothesized relationship between the $C^n \times T_{90}$ value and the patients' response. Similar calculations were done for the optimized regimen. For an n value of 1.24, the optimized regimen gave a 76% cure for $T_a$ and $T_1$ tumors. This translates into a 20% (76% minus 56%) increase in cures by using optimized treatment as opposed to standard treatment. For an n value of 1, the percentage of $T_a$ tumors that were exposed to the $C^n \times T_{90}$ remained at 78% (Table 1), whereas the corresponding proportion of $T_1$ tumors was lower at 17%, and the calculated improvement in treatment outcome was 18%.

## Discussion

Our previous clinical and basic pharmacology studies on the use of MMC in intravesical chemotherapy have identified several factors that may enhance intravesical MMC treatment efficacy. The present study incorporates the pharmacologic data and the use of computer simulations to depict the effect of changing the treatment parameters and the environmental factors on the expected therapeutic outcome. An optimized regimen was selected based on the simulation results and practical considerations as follows.

### *Dose and instillation time*

The most significant enhancement was achieved when the dose was increased from 20 to 40-mg. Because of the limited increase in drug exposure for a further increase of the dose to 60-mg, the limited clinical experience with this dose, and the high cost of medication, we proposed to use the 40-mg dose in the optimized regimen. The doubling of the instillation time to 4-h gave only a slight improvement. Hence, a 2-h instillation time was selected for practicality and for patient convenience and compliance.

### *Urine pH*

The urine pH may alter the treatment outcome in three ways: it may affect the absorption, stability, or cytotoxicity of MMC. We have shown that drug absorption via an intact urothelium is insignificant (~3% of the dose [6]). Furthermore, because MMC ($pK_a$, 2.8) is mainly unionized at the normal urine pH of 5–8, the effect of urine pH on absorption will be minor. MMC is usually given unbuffered; hence, the pH of the bladder instillate will approach that of the urine produced during therapy. The urine pH has a significant effect on drug degradation. The degradation of MMC in human urine was 10-fold greater at pH 5 than at pH 7, and nonenzymatic degradation of MMC accounted for more than one-half of the drug loss at low pH [6]. A previous study conducted by other investigators [13] shows that in monolayer cultures of human bladder cell lines studied in a pH range of 5.2–9.7, the cytotoxicity of MMC was enhanced in media with a pH of <8.

We examined the effect of pH on the MMC sensitivity of human bladder tumor explants that have a ~150-cell diameter. The labeling index of the tumor cells in the untreated controls was unchanged for pH 5, 6, and 7.4, indicating that the 2-h exposure at the acidic pH had no adverse effect on cell viability. The MMC activity in the explants was not affected by pH; the drug concentrations and exposure times needed to inhibit tumor cell proliferation at pH 5, 6, and 7.4 were similar (manuscript in preparation). These data suggest that the enhancement of MMC activity at lower pH observed in monolayer cultures did not take place in the multicellular human solid tumors. This difference may be due to the differences in the two experimental models. Monolayers are homogeneous, whereas the tumor explants are heterogeneous with respect to cell type, oxygenation status, regional pH, and drug penetration. These heterogeneities may have disguised the pH-dependent sensitivity to MMC. It is also possible that the tumor cells in the explants did not assume the pH of the buffer. Obviously, the pH in multicellular systems will be more difficult to manipulate than that in monolayer systems.

**Table 2.** Conditions of standard and pharmacology-based treatment regimens

| | Standard regimen | Pharmacology-based regimen |
|---|---|---|
| Dose | 20 mg in 20 ml water | 40 mg in 20 ml water |
| Urine production | Not controlled | No fluid and no caffeine intake overnight and during treatment |
| Urine pH | Not controlled | $NaHCO_3$, 1,300 mg at night and before treatment |
| Residual volume | Measured by sonography, not controlled | Complete voiding, confirmed by sonography |
| Duration of instillation | 2 h | 2 h |
| Number of treatments | Weekly × 6 | Weekly × 6 |

Another consideration that does not support the use of a lower pH during drug instillation is that tumors that are embedded in the bladder wall are perfused by the blood circulation and are subjected to physiologic pH regulation. Lowering the pH of the dosing solution will not improve the activity of MMC in these tumors. On the basis of the pH dependence observed for MMC stability and the lack of pH dependence noted for MMC activity in human bladder tumor explants, we decided to control the urine pH at about 7.

*Volumes of urine and dosing solution*

An increased urine volume will unfold the bladder, increase the surface area, and reduce the thickness of the distensible bladder wall. Distension of the bladder may have several effects on the drug concentration in the deeper tissues. First, more drug passes through the urothelium, which is thinner and has a bigger surface area, resulting in increased tissue concentration. Second, distension of the bladder may reduce the tissue blood flow [18, 20] and, hence, decrease the amount of drug removed by the circulation. In rats given 0.35 or 0.6 ml doxorubicin at the same concentration of 4 mg/ml by intravesical administration, the serum drug concentrations were 70%–400% higher in the group given the larger volume of drug solution [9]. In a preliminary study, dogs with highly distended bladders showed a 6-fold higher plasma concentration and a 7-fold higher tissue concentration [30]. However, distension of patients' bladders during MMC instillation would require an instillation volume in excess of 200 ml and an MMC dose of more than 100 mg to give a sufficiently high concentration. This may be impractical in view of the potential toxicity and the medication costs.

In current clinical practice, MMC is given in a volume of 20–40 ml. A recent study in patients suggests that the instillation volume may also affect the drug's contact to the lower half of the posterior wall of the bladder adjoining the trigone, which is usually folded by abdominal pressure [15]. A volume of 40 ml was needed to expand the area and therefore allow the drug solution to come into contact with the epithelial surface. On the other hand, administration of the same dose in a smaller volume will result in an increased urine concentration. Since this concentration is the driving force for diffusion into the bladder wall, reducing the volume of administration will result in increased bladder-wall concentrations. Furthermore, data obtained in dogs showed that there was no difference in the concentrations achieved in the tissues either in contact or not in contact with the drug solution (unpublished observation). The recent SWOG trial comparing MMC and bacillus Calmette-Guérin used an instillation volume of 20 ml. On the basis of these data, an instillation volume of 20 ml was selected for both the optimized and the standard regimens.

*Statistical considerations*

To detect a 20% improvement, approximately 88 evaluable patients per arm are needed for a 5% level of significance and 80% power [4, 5]. To evaluate an additional therapeutic endpoint, i.e., an increase in the time to recurrence, a larger sample size of 116 patients per treatment arm is needed. The larger sample size also provides a margin in case the improvement is slightly less than the projected 20% due to unforeseen factors. Note that the 20% improvement is achieved by optimizing several parameters. A single change in the dose from 20 to 40 mg would increase the exposure of $T_a$ patients to the $C^n \times T_{90}$ from 22% to 56%, whereas $T_1$ patients would not yet receive the $C^n \times T_{90}$. This corresponds to a 64% cure or an 8% (64%–56%) improvement. To detect an 8% improvement, more than 450 patients per arm are needed. On the basis of these data and projections, we speculate that the inability of previous clinical studies comparing 20- and 40-mg doses to demonstrate a significant benefit for the higher dose may have been due to an inadequate sample size for the detection of a relatively small improvement [25].

*Potential errors in the projected treatment outcome*

For the simulations, an intact urothelium with a drug-concentration gradient ranging from 36 to 1 across the urothelium is assumed based on studies in radical cystectomy patients [29]. Equations 2 and 3 predict that increases in urine concentrations will lead to proportional increases in concentrations in the urothelium and the bladder wall. This prediction is supported by a separate study in rabbits [30]. This proportionality could be disturbed if MMC enhances its own absorption by irritation of the bladder wall. Local irritation is a known effect of MMC [24] and would result in higher than expected bladder-wall concentrations. Furthermore, our previous studies indicated a disruption of the barrier function of the urothelium for several days after surgery, leading to enhanced MMC penetration into the bladder wall and the systemic circulation during the first treatment. This effect was diminished after 1 week and was not seen during the subsequent five

weekly treatments [6]. The high tissue concentration during the first treatment may produce a greater improvement in the treatment outcome. Furthermore, studies in radical cystectomy patients showed a higher MMC concentration in tumor tissues than in the adjacent normal tissue [29]. It is possible that the projected treatment efficacy is underestimated.

*Efficacy trial*

In summary, the simulation projected an 18%–20% improvement in the treatment outcome for the optimized regimen over the currently used standard treatment. An efficacy trial to compare the standard and optimized treatments has been initiated (Table 2).

The present data show the use of pharmacologic data and computer simulations to rank-order the relative significance of several treatment conditions, to select the important parameters for optimization, and to project the outcome of the optimized regimen as well as the test sample size. We consider these features to be noteworthy because in the field of experimental therapeutics it is not always possible to change methodically the parameters used to predict the therapeutic outcome; many trials are conducted on a trial-and-error basis. The simulated data showed that alterations in four parameters were needed to achieve a 20% improvement and that alterations in fewer parameters would give a less significant improvement. By this approach, trials of less efficacious regimens can be avoided.

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