Research Article

Bladder Tissue Pharmacokinetics of Intravesical Mitomycin C and Suramin in Dogs

Leijun Hu,¹ M. Guillaume Wientjes,^{1,2} Jing Li,¹ and Jessie L.-S. Au^{1,3}

Received 14 May 2010; accepted 22 June 2010; published online 13 July 2010

Abstract. Suramin, at non-cytotoxic doses, reverses chemoresistance and enhances the activity of mitomycin C (MMC) in mice bearing human bladder xenograft tumors. The present study evaluated the pharmacokinetics of the intravesical suramin and MMC, alone or in combination, in dogs. Animals received either high dose suramin (20 mg/ml), low dose suramin (6 mg/ml), MMC (2 mg/ml), or combination of low dose suramin and MMC, instilled for 2 h. The dosing volume was 20 ml. All groups showed dilution of drug levels over time due to continued urine production. For single agent suramin, the results showed (a) 5% to 10% penetration into bladder tissues, (b) minimal and clinically insignificant systemic absorption (i.e., undetectable at low dose or a peak concentration that was 6,000× lower than urine concentrations), and (c) disproportionately higher drug penetration and concentrations in bladder tissues at the higher dose. Results for single agent MMC are consistent with our earlier observations. The co-administration of MMC did not alter the plasma, urine, or tissue pharmacokinetics of suramin. Adding suramin did not alter plasma or tissue pharmacokinetics of MMC, but lowered the MMC concentrations in urine by about 20%. This may be in part due to accelerated MMC degradation by co-incubation of suramin or due to variations in urine production rate (because animals were allowed for water during treatment). Suramin readily penetrates the urothelium and into deeper bladder tissues, indicating its potential utility in intravesical therapy.

KEY WORDS: administration; intravesical; mitomycin; suramin; urinary bladder neoplasms.

INTRODUCTION

Intravesical chemotherapy is used to reduce the recurrence of non-muscle invading bladder cancer (1,2). We have shown that the variable and incomplete response in patients can be attributed to insufficient drug delivery to tumor cells and tumor resistance to chemotherapeutic agents (3–5). We further showed in a phase III trial that pharmacokinetic interventions can maximize the mitomycin C (MMC) delivery to bladder tissues and improve treatment efficacy (e.g., increasing the 5-year recurrence-free rate from 25% to 41%) (3). Our second strategy is to overcome tumor resistance to chemotherapy, for which we have introduced the concept of using non-cytotoxic doses of suramin as a chemosensitizer, in *ex vivo* and *in vivo* preclinical models (6–8). We have further investigated suramin, at doses that yielded the non-cytotoxic plasma concentrations of 10–50 µM in plasma in four phase I/II trials in chemotherapy-naïve, -pretreated, or -refractory patients with nonsmall cell lung cancer, breast cancer, and renal cell cancer (9–12). These data have led to two randomized trials being initiated in lung cancer (11,12). Additional preclinical data in patient bladder tumor

histocultures and mice bearing RT4 transitional cell carcinoma indicate non-cytotoxic suramin enhances the efficacy of MMC (13). Hence, clinical evaluation of combination intravesical therapy with MMC and suramin is of interest.

The results of two phase I trials evaluating intravesical therapy with suramin alone show that it can be safely administered to patients between 100 and 150 mg/ml (14,15). The goal of these earlier trials was to use suramin as the cytotoxic therapeutic. In contrast, we are interested in using suramin as a sensitizer. For this effect, suramin shows an unusual dose response; benefits were observed only at the non-cytotoxic concentrations of 10–50 µM and were abolished at higher concentrations (8,11). Hence, there is a need to define the relationship between the dose and the drug concentrations in bladder tissues for intravesical suramin and to evaluate possible pharmacokinetic interactions between suramin and MMC, prior to embarking on a clinical study of the MMC/suramin combination. This was addressed by the present study. Dogs were used as the animal model due to the similarity of the anatomy and thickness of the dog bladders to human bladders and because intravesical administration can be readily performed in dogs (16–18).

MATERIALS AND METHODS

Chemicals and Reagents

Suramin, trypan blue, tetrabutylammonium bromide, and tetrabutylammonium hydrogensulfate were obtained

¹ College of Pharmacy, The Ohio State University, 500 West 12th Avenue, Columbus, Ohio 43210, USA.

² Comprehensive Cancer Center, The Ohio State University, 500 West 12th Avenue, Columbus, Ohio 43210, USA.

³ To whom correspondence should be addressed. (e-mail: au.1@osu.edu)

from Sigma Chemical Co. (St. Louis, Missouri), HPLC grade reagents from Fisher Scientific (Fair Lawn, New Jersey), MMC from Bristol Myer Squibb Co. (Wallingford, Connecticut), and porfiromycin from National Cancer Institute (Bethesda, Maryland). All reagents were used as received.

Animal Protocols

The protocol was approved by the Institutional Animal Care and Use Committee of the Ohio State University. The study used 18 male or female beagle dogs, donated by Battelle Memorial Institute (Columbus, Ohio) and Springborn Laboratory (Springfield, Ohio) and weighing 10.5 ± 1.4 kg (mean $\pm$ SD). Animals received either single agent suramin at high dose (20 mg/ml, $n=3$) or low dose (6 mg/ml, $n=5$), single agent MMC (2 mg/ml, $n=4$), or the combination of low dose suramin plus MMC ($n=5$). Dosing solutions (20 ml) were prepared in physiologic saline. Procedures were as previously described (18). Briefly, animals were fasted overnight with free access to water. Animal were anesthetized with isoflurane inhalation. Urinary catheters were used for dose instillation and urine sampling and jugular vein catheters for blood sampling. Immediately before treatment, the urinary bladder was emptied and rinsed thrice with normal saline. Animals showing blood in urine were regarded as having a damaged urothelium and were excluded. Drug solution was instilled intravesically for 2 h and the catheters were kept closed during this period. Serial urine and blood samples were obtained. After treatment, the bladder was surgically excised, drained of its content, and cut into three pieces (left and right lateral sides, dome). Tissue sections were snap-frozen in liquid nitrogen. Tissue removal and freezing was completed within 2 min after draining the bladder content or within 5 min after surgical incision of the abdomen wall.

Tissue Sample Processing

Bladder pieces were cryo-sectioned into a series of 20- μ m thick slices, parallel to the urothelial surface, as described (18). To avoid drug contamination on tissue sections, the first two section slices were discarded. Extraction of MMC or suramin from bladders after treatments with single agents used previously described methods (12, 16).

For animals treated with both suramin and MMC, tissues were first extracted for MMC. The residual tissues were then extracted for suramin. A pilot study comparing the suramin extraction yield in tissue homogenates with and without a prior extraction for MMC established that these procedures yielded 92–96% recovery of suramin in tissue, indicating near-complete suramin recovery after the sequential extraction procedures.

Other Methodologies

Extraction of suramin or MMC in plasma and urine samples, as well as analysis of drug concentrations by high performance liquid chromatography, were as previously described (12, 16).

MMC Stability in Urine in the Presence Suramin

Suramin (6 mg/ml) and MMC (2 mg/ml) were added to dog urine (collected from dogs not in the study) and

incubated at 37°C. Samples were withdrawn and analyzed for pH values and drug concentrations.

Pharmacokinetic Data Analysis

Urine concentration–time profiles and tissue concentration–depth profiles were analyzed using Eqs. 1 and 2, respectively. These equations have been used successfully for several other drugs (16,18).

$$C_u = \frac{\text{Dose}}{V_u} e^{-(k_a+k_d)t}, \text{ where } V_u = V_0 + k_0t + V_{\text{res}} \quad (1)$$

$$C_x = (C_0 - C_b) e^{\frac{-0.693}{W_{1/2}}(x-50)} + C_b \quad (2)$$

C_u is urine concentration at time t . (k_a+k_d) is a hybridized constant describing drug adsorption and degradation during treatment. V_u is urine volume at time t and is the sum of the dosing solution volume (V_0), residual urine volume at time of dose instillation (V_{res}), and urine produced during treatment (k_0t). k_0 is the zero order urine production rate. C_x is drug concentration in the capillary-perfused submucosa and muscularis at distance x from the urothelial surface. Concentration decline over the urothelium, from 0 to 50 μ m in dog bladders, is presumed to be linear with depth due to absence of capillaries (and hence the first order drug removal by blood flow). C_b is average drug concentration of the blood perfusing the capillary-rich tissue. Half width ($W_{1/2}$) is the distance by which drug concentration drops 50%. The average tissue concentration (C_{ave}) was calculated as (total drug amount in bladder tissue) divided by (total tissue weight).

Statistical Analysis

Comparison of values between groups was performed using ANOVA for comparisons between three or more groups, or two-tailed Student's t tests for two groups.

RESULTS

Plasma Pharmacokinetics of Intravesical Single Agent Suramin in Dogs

Animals treated with low dose suramin (6 mg/ml) showed undetectable plasma drug concentrations (<1 μ g/ml) at all times. The high dose group showed increasing plasma concentrations to reach the maximum level of 2.53 ± 0.14 μ g/ml (mean $\pm$ SD) at the end of the 2-h instillation (Fig. 1). This concentration was $>6,000\times$ lower compared to the urine concentration at 2 h (Fig. 2).

Urine Pharmacokinetics of Intravesical Single Agent Suramin in Dogs

Urine concentrations declined with time continuously during the 2-h experiment, due to urine production (Fig. 2). Table I summarizes the urine pharmacokinetic parameters. Compared to the high dose group, the increase in urine volume was higher in the low dose group, i.e.,

Fig. 1. Plasma concentrations of suramin and MMC during intravesical treatment. Dogs received suramin, MMC, or both, by intravesical installation. Animals received single agent suramin at two doses, low dose (120 mg/20 ml or 6 mg/ml, $n=5$) or high dose (400 mg/20 ml or 20 mg/ml, $n=3$); single agent MMC (40 mg/20 ml, $n=4$) or combination of low dose suramin and MMC ($n=5$). Data are mean and 1 SD. Plasma samples collected at 5 min in the single agent high dose suramin group, and all time points in treatments using the low dose suramin had undetectable suramin concentrations (i.e., below the detection limit of 1 µg/ml). Note the logarithmic scale

50% vs 12%, which corresponded to the greater dilution of urine concentration in the low dose group (58% vs 80% of the initial concentrations in the dosing solutions). The reasons are unclear. The dose fractions recovered in the 2-h urine were $90.7 \pm 1.6\%$ in the low dose group and $95.7 \pm 6.3\%$, in the high dose group, indicating less than 10% of the dose was degraded or absorbed.

Bladder Tissue Pharmacokinetics of Intravesical Single Agent Suramin in Dogs

Figure 3 shows the tissue concentration–depth profiles of suramin, and Table II summarizes the tissue pharmacokinetic parameters. The half width of tissue concentration decline was about 300 µm. The ratio of urothelial to urine drug concentration (i.e., C_{uro}/C_u ratio), which gives a measure of the partition of suramin across the urothelium, was significantly higher in the high dose group compared to the low dose group ($\sim 10\%$ vs $\sim 5\%$, $p < 0.05$). The same trend was observed for the ratio between the average tissue concentration and the urine concentration (i.e., C_{ave}/C_u ratio, which is a measure of the extent of tissue penetration). Together, these data indicate disproportionately higher penetration and absorption of suramin into the bladder wall at higher urine concentrations. The lower absorption of the low dose is in agreement with the undetectable plasma concentrations in this group (i.e., < 1 µg/ml). No differences were observed in the other tissue pharmacokinetic parameters.

Plasma, Urine, and Bladder Tissue Pharmacokinetics of Intravesical MMC in Dogs

Figures 1, 2, and 3 show the plasma, urine, and bladder tissue concentrations, respectively, after intravesical MMC. The pharmacokinetic parameters are summarized in Table II.

Plasma concentrations reached an early maximum at approximately 15 min, followed by essentially steady concentrations. Urine concentrations show an initial drop from the dosing solution concentration of 2 mg/ml due to dilution in the residual urine, followed by a slow concentration decline due to dilution in the newly formed urine, and drug absorption and degradation. Tissue concentrations declined approximately 30-fold over the urothelium ($C_{uro}/C_u \sim 3.3\%$), followed by a log-linear decline with depth as previously observed [16]. The steepness of the decline was greater than previously observed [16]; the reasons are not apparent.

Potential Pharmacokinetic Interactions Between Intravesical Suramin and Intravesical MMC

The results are shown in Figs. 1, 2, and 3 and Tables I and II.

For suramin, there were no differences in the urine and tissue levels in the absence or presence of MMC. Similarly, the plasma suramin concentration remained below the detection limit of 1 µg/ml, with or without MMC. These results indicate that addition of MMC did not significantly alter the urine and plasma pharmacokinetics of suramin.

For MMC, the addition of suramin produced a relative minor, but statistically significant, decrease in the recovery of MMC in urine at 2 h (from $87.4 \pm 6.8\%$ to $78.9 \pm 6.6\%$, $p < 0.05$). This difference was also reflected in the urine concentration–time profiles with slightly lower concentrations in the combination group, partly explained by differences in residual volume and urine production rate. Urine pH was measured in some of the dogs, but did not show differences between groups. This small difference in urine concentration–time profiles did not affect the MMC absorption into tissues or blood, as indicated by the nearly superimposable plasma and tissue concentration–depth profiles for single agent MMC or MMC/suramin combination.

Fig. 2. Urine concentration–time profiles of suramin and MMC during intravesical treatments. Dogs received suramin, MMC, or both, by intravesical installation. Animals received single agent suramin at two doses, low dose (120 mg/20 ml or 6 mg/ml, $n=5$) or high dose (400 mg/20 ml or 20 mg/ml, $n=3$); single agent MMC (40 mg/20 ml, $n=4$) or combination of low dose suramin and MMC ($n=5$). Data are mean and 1 SD. Note the linear scale

Table I. Urine Pharmacokinetics of Suramin and MMC

Groups		C_u ($\mu\text{g/ml}$)	V_{res} (ml)	k_0 (ml/min $\times 10^{-2}$)	$k_a + k_d$ ($\text{min}^{-1} \times 10^{-4}$)	V_{final} (ml)	IUV (%)
A. Suramin							
Group 1: High dose suramin ($n=3$)	Range	15.6–17.4	1.12–1.66	0.73–2.85	0–2.38	22–25	4.1–15.8
	Median	16.5	1.59	2.79	0.0001	25	15.4
	Mean $\pm$ SD	$16.5 \pm 0.89^*$	1.46 ± 0.29	2.12 ± 1.21	$0.79 \pm 1.37^{**}$	24 ± 2	11.8 ± 6.6
Group 2: Low dose suramin without MMC ($n=5$)	Range	2.27–3.74	0.32–5.33	3.89–17.2	5.34–22.0	30–45	18.4–85.1
	Median	3.28	2.51	8.90	10.70	31	52.5
	Mean $\pm$ SD	3.07 ± 0.68	2.78 ± 2.05	9.52 ± 4.95	12.8 ± 6.5	34 ± 6	50.3 ± 24.3
Group 3: Low dose suramin with MMC (suramin, $n=5$)	Range	3.10–4.44	2.18–3.96	2.35–10.7	2.85–19.1	25–35	12.7–57.8
	Median	3.41	3.66	9.20	8.30	35	46.1
	Mean $\pm$ SD	3.60 ± 0.51	3.17 ± 0.91	7.35 ± 3.50	8.82 ± 6.47	32 ± 5	38.1 ± 18.4
B. MMC							
Group 4: MMC without low dose suramin ($n=4$)	Range	1.04–1.54	0–3.04	6.62–11.3	0.47–27.2	25–35	8.5–62.9
	Median	1.19	1.77	7.48	22.0	30	43.0
	Mean $\pm$ SD	1.24 ± 0.23	1.64 ± 1.27	6.96 ± 4.03	15.9 ± 11.6	30 ± 4	39.4 ± 23.3
Group 3: MMC with low dose suramin ($n=5$)	Range	0.76–1.30	2.18–3.96	2.35–10.7	3.56–21.4	25–35	12.7–57.8
	Median	0.82	3.66	9.20	16.7	35	46.1
	Mean $\pm$ SD	0.96 ± 0.24	3.17 ± 0.91^a	7.35 ± 3.50^a	14.8 ± 6.9	32 ± 5	38.1 ± 18.4

Dogs received intravesical treatment as described in the legend of Fig. 1. The urine concentration–time profiles were analyzed using Eq. 1. Differences between group means were evaluated by ANOVA for comparisons between all groups (V_{res} , k_0 , V_{final}) or for more than two groups ($k_a + k_d$ for suramin). Comparisons of sets of two groups used Student's two-tailed t test. Groups that show significant differences are noted by asterisks

C_u urine concentration at 120 min, V_{res} residual volume of urine at the time of dosing, k_0 the urine production rate, $k_a + k_d$ the first order rate constant for drug absorption into the bladder and degradation in urine, V_{final} total urine volume at 120 min, IUV increase in urine volume during treatment

* $p < 0.05$, C_u for Group 1 was higher than for Groups 2 and 3 (ANOVA with post hoc Tukey test). This is expected due to the higher dose.

** $p < 0.05$, $k_a + k_d$ for Group 1 was lower than for Group 2 and 3 (ANOVA with post hoc Tukey test)

^a Group 3: values for V_{res} and k_0 were calculated by fitting of suramin concentration data

MMC Stability in Suramin-Containing Dog Urine

To investigate MMC degradation as possible cause of the lower urine concentrations in the presence of suramin, we determined the concentration decline and changes in urine pH because we have shown that MMC is unstable in acidic urine (19). After incubation of the suramin MMC combination in

urine for 2 h *in vitro*, the urine pH decreased from 7.0 to 6.2 and the amount of MMC declined to $91.7 \pm 4.10\%$ ($n=5$) of the initial amount.

DISCUSSION

The goals of the present study were to evaluate the ability of suramin to penetrate bladder tissue during intravesical therapy and the extent of such penetration and to evaluate the feasibility of using suramin as a sensitizer with MMC. The major results are as follows.

Intravesical Suramin Yields Appreciable Concentrations in Bladder Tissues

Urothelium is the major barrier to drug absorption into the bladder wall. Compounds with high lipophilicity (e.g., paclitaxel with an octanol/water partition coefficient >99) show more favorable urothelial penetration compared to compounds with lower lipophilicity (e.g., MMC, doxorubicin and 5-fluorouridine, with octanol/water partition coefficients of $\#0.5$) (20). This observation is consistent with passive diffusion as the major transport mechanism through the urothelium. Suramin, a polysulfonated naphthylurea with six sulfonate groups, is highly polar with a lower lipophilicity (octanol/water partition coefficient is 0.00032 (21) and, hence, would be expected to have low penetration across the urothelium. Surprisingly, the results of the present study indicate appreciable drug penetration; the respective C_{uro}/C_u ratio, C_{uro} (concentration in the urothelium), and C_{ave} (average bladder tissue concentra-

Fig. 3. Tissue–depth profiles of suramin and MMC. Dogs received suramin, MMC, or both, by intravesical installation. Animals received single agent suramin at two doses, low dose (120 mg/20 ml or 6 mg/ml, $n=5$) or high dose (400 mg/20 ml or 20/ml, $n=3$); single agent MMC (40 mg/20 ml, $n=4$) or combination of low dose suramin and MMC ($n=5$). Data are mean and 1 SD. For MMC and low dose suramin, several animals showed undetectable levels at depth beyond 1,200 μm . Hence, the profiles were limited to the first 1,200 μm . Note the logarithmic scale

Table II. Tissue Pharmacokinetic Parameters of Suramin and MMC

Groups		C_{uro} ($\mu\text{g/g}$)	C_{ave} ($\mu\text{g/g}$)	C_b ($\mu\text{g/g}$)	C_{uro}/C_u (%)	C_{ave}/C_u (%)	$W_{1/2}$ (μm)
A. Suramin							
Group 1: High dose suramin ($n=3$)	Range	1,222–2,684	90.1–506	0–35.1	7.30–15.4	0.58–4.33	250–728
	Median	1,525.8	451	0.849	9.75	2.82	320
	Mean $\pm$ SD	1,811 $\pm$ 772*	349 $\pm$ 226*	12.0 $\pm$ 20.0	10.8 $\pm$ 4.2	2.58 $\pm$ 1.89	432 $\pm$ 258
Group 2: Low dose suramin without MMC ($n=5$)	Range	35.4–641	23.7–73.9	0–7.86	1.91–17.1	0.86–2.23	110–643
	Median	97.9	37.6	0	3.94	1.21	316
	Mean $\pm$ SD	166 $\pm$ 174	41.6 $\pm$ 15.6	1.84 $\pm$ 2.67	5.09 $\pm$ 4.40	1.44 $\pm$ 0.55	316 $\pm$ 152
Group 3: Low dose suramin with MMC ($n=5$)	Range	56.1–354	24.8–113.2	0–10.4	1.66–9.65	0.54–2.55	100–1,097
	Median	115	44.8	3.56	3.10	1.33	287
	Mean $\pm$ SD	147 $\pm$ 87	50.1 $\pm$ 23.8	3.60 $\pm$ 0.47	4.01 $\pm$ 2.19	1.40 $\pm$ 0.60	380 $\pm$ 264
B. MMC							
Group 4: MMC without low dose suramin ($n=4$)	Range	8.53–82.9	2.48–25.8	0–2.71	0.81–7.96	0.19–2.48	100–342
	Median	31.6	5.66	0.40	3.17	0.45	207
	Mean $\pm$ SD	37.2 $\pm$ 27.9	6.95 $\pm$ 5.76	0.74 $\pm$ 0.79	3.27 $\pm$ 2.27	0.65 $\pm$ 0.63	216 $\pm$ 91
Group 3: MMC with low dose suramin ($n=5$)	Range	9.67–72.2	3.14–17.4	0.09–6.34	0.74–9.41	0.24–2.13	100–297
	Median	30.9	5.97	1.11	3.48	0.59	162
	Mean $\pm$ SD	38.1 $\pm$ 20.7	7.00 $\pm$ 4.52	1.68 $\pm$ 1.70	4.30 $\pm$ 2.73	0.77 $\pm$ 0.51	178 $\pm$ 74

Parameters were calculated using Eq. 2. Differences between group means were evaluated by ANOVA for comparisons between more than two groups (Groups 1, 2, and 3 for suramin). Comparisons of sets of two groups used Student's two-tailed t test. Groups that show significant differences are noted by asterisks

C_{ave} average tissue concentration, C_{uro} drug concentration in the urothelium (at 50 μm depth), C_b drug concentration in the deep muscle layer, C_u final urine concentration, $W_{1/2}$ (half width) tissue distance over which C declines by 50%

* $p < 0.05$, C_{uro} and C_{ave} for Group 1 were higher than for Groups 2 and 3 (ANOVA with post hoc Tukey test). This is expected due to the higher dose

tion) were $\sim 5\%$, $\sim 170 \mu\text{g/g}$ ($\sim 120 \mu\text{M}$), and $\sim 40 \mu\text{g/g}$ ($\sim 30 \mu\text{M}$) at the 6 mg/ml dose and $\sim 10\%$, $\sim 1,700 \mu\text{g/g}$ ($\sim 1,200 \mu\text{M}$), and $\sim 420 \mu\text{g/g}$ ($\sim 300 \mu\text{M}$) at the 20 mg/ml dose. These results indicate a nonlinear, dose-dependent drug penetration, with disproportionately higher penetration at the higher dose. In addition, the C_{uro}/C_u ratio for suramin was two to three times higher compared to MMC, doxorubicin, and 5-fluorouridine (5–10% *vs* $\sim 3\%$) (16,18,22).

Two possible reasons for the higher bladder tissue penetration of suramin are differences in transport mechanism(s) and drug retention in tissues. With respect to transport, we reported a linear, dose-independent increase for MMC, consistent with passive diffusion as the primary transport mechanism (16). Additional facilitated or active transport mechanisms would increase the penetration across the urothelium. For example, suramin can enter cells by endocytosis or pinocytosis (23). The same in urothelial cells would explain the higher penetration. Of interest is the observation of the significant increase in suramin absorption with dose (i.e., $2\times$ higher C_{uro}/C_u when the dose was increased from 6 mg/ml to 20 mg/ml). In contrast, the penetration of MMC was not altered when the dose was increased from 1 to 2 mg/ml (16). The reasons for the dose-dependent urothelial penetration by suramin are not apparent.

With respect to retention, we reported that drugs with insignificant tissue binding such as MMC rapidly equilibrate with bladder tissues and reach steady state equilibrium within minutes (18), whereas drugs such as paclitaxel that are tightly bound to tissues or proteins are retained in bladder tissues (20, 24). Suramin is highly protein-bound to plasma proteins ($>99.7\%$), with albumin accounting for 80–90% of the total binding proteins (25,26). Based on the high albumin concentration in interstitial fluid ($\sim 80\%$ of that in plasma (27), high

protein-binding of suramin is expected and would explain the higher C_{uro}/C_u ratios.

Applying Bladder Tissue Pharmacokinetics of Intravesical Suramin to Calculate the Tissue Levels for Previous Treatments in Patients

Two phase I clinical trials have employed intravesical doses of 100–150 mg/ml suramin as a single agent cytotoxic (1,2,14,15). To evaluate whether these doses deliver sufficient drug levels to cause cytotoxicity, we used the bladder tissue pharmacokinetic parameters established in the current study (for the 20 mg/ml dose) to estimate the corresponding tissue concentrations. Assuming no further increases in the C_{uro}/C_u ratios at the higher doses used in humans, we calculated, at the time of dose instillation, the C_{uro} would be $\sim 7 \text{ mM}$ and $\sim 10 \text{ mM}$ and the C_{ave} would be ~ 2 and $\sim 2.5 \text{ mM}$, for the 100 and 150 mg/ml dose, respectively. These levels are ~ 10 -folds higher than the 175–280 μM concentrations that cause cytotoxicity in cultured cells (28).

Pharmacokinetic Interactions Between Intravesical MMC and Suramin

The present results show that MMC did not alter the plasma, urine, or bladder tissue pharmacokinetics of intravesical suramin. Adding suramin to MMC decreased the urine concentrations and dose fraction of MMC remaining at 2 h. However, these changes, while statistically significant, were relatively minor ($<10\%$) and did not result in significant changes in the tissue pharmacokinetics of MMC or its absorption into the systemic circulation.

Potential Use of Intravesical Suramin to Enhance the Activity of Intravesical MMC

Activity of suramin as a chemosensitizer is observed at extracellular concentrations between 10 and 50 μM and is abolished at higher concentrations. Based on the results of the present study, the low dose of 6 mg/ml yielded 100 μM in the urothelium (50 μm depth) and average concentration of 35 μM in bladder tissue. Hence, clinical evaluation of suramin as a sensitizer in intravesical therapy should use starting doses lower than 6 mg/ml, e.g., 3 mg/ml. An added advantage of using these low doses is the minimal suramin systemic exposure of less than 1 $\mu\text{g}/\text{ml}$. Finally, in view of the long retention of suramin in tissues, e.g., up to >12 weeks in rats (29), a routine weekly dose schedule would cause drug accumulation. Additional studies to address the post-treatment suramin retention in bladder tissues and the dosing frequency are warranted.

CONCLUSIONS

Upon intravesical instillation, suramin penetrated the urothelium and yielded appreciable drug concentrations in bladder tissues, in a dose-dependent manner (increasing with higher doses). There are no or minimal pharmacokinetic interactions between suramin and MMC, indicating the feasibility of combining intravesical suramin with intravesical MMC.

ACKNOWLEDGMENTS

This work was supported in part by research grants R01CA93871 and R37CA49816 from NCI, NIH, and DHHS.

REFERENCES

- Smaldone MC, Gayed BA, Tomaszewski JJ, Gingrich JR. Strategies to enhance the efficacy of intravesical therapy for non-muscle invasive bladder cancer. *Minerva Urol Nefrol*. 2009;61(2):71–89.
- Perabo FG, Muller SC. New agents in intravesical chemotherapy of superficial bladder cancer. *Scand J Urol Nephrol*. 2005;39(2):108–16.
- Au JL, Badalament RA, Wientjes MG, Young DC, Warner JA, Venema PL, *et al*. Methods to improve efficacy of intravesical mitomycin C: results of a randomized phase III trial. *J Natl Cancer Inst*. 2001;93(8):597–604.
- Wientjes MG, Badalament RA, Au JL. Use of pharmacologic data and computer simulations to design an efficacy trial of intravesical mitomycin C therapy for superficial bladder cancer. *Cancer Chemother Pharmacol*. 1993;32(4):255–62.
- Shen Z, Shen T, Wientjes MG, O'Donnell MA, Au JL. Intravesical treatments of bladder cancer: review. *Pharm Res*. 2008;25(7):1500–10.
- Song S, Wientjes MG, Gan Y, Au JL. Fibroblast growth factors: an epigenetic mechanism of broad spectrum resistance to anticancer drugs. *Proc Natl Acad Sci USA*. 2000;97(15):8658–63.
- Song S, Wientjes MG, Walsh C, Au JL. Nontoxic doses of suramin enhance activity of paclitaxel against lung metastases. *Cancer Res*. 2001;61(16):6145–50.
- Zhang Y, Song S, Yang F, Au JL, Wientjes MG. Nontoxic doses of suramin enhance activity of doxorubicin in prostate tumors. *J Pharmacol Exp Ther*. 2001;299(2):426–33.
- George S, Dreicer R, Au JLL, Shen T, Rini BI, Roman S, *et al*. Phase I/II trial of 5-fluorouracil and a noncytotoxic dose level of suramin in patients with metastatic renal cell carcinoma. *Clin Genitourinary Cancer*. 2008;6(2):79–85.
- Lam ET, Au JL, Otterson GA, Guillaume WM, Chen L, Shen T *et al*. Phase I trial of non-cytotoxic suramin as a modulator of docetaxel and gemcitabine therapy in previously treated patients with non-small cell lung cancer. *Cancer Chemother Pharmacol*. 2010.
- Villalona-Calero MA, Otterson GA, Wientjes MG, Weber F, Bekaii-Saab T, Young D, *et al*. Noncytotoxic suramin as a chemosensitizer in patients with advanced non-small-cell lung cancer: a phase II study. *Ann Oncol*. 2008;19(11):1903–9.
- Villalona-Calero MA, Wientjes MG, Otterson GA, Kanter S, Young D, Murgo AJ, *et al*. Phase I study of low-dose suramin as a chemosensitizer in patients with advanced non-small cell lung cancer. *Clin Cancer Res*. 2003;9(9):3303–11.
- Xin Y, Lyness G, Chen D, Song S, Wientjes MG, Au JL. Low dose suramin as a chemosensitizer of bladder cancer to mitomycin C. *J Urol*. 2005;174(1):322–7.
- Ord JJ, Streeter E, Jones A, Le Monnier K, Cranston D, Crew J, *et al*. Phase I trial of intravesical suramin in recurrent superficial transitional cell bladder carcinoma. *Br J Cancer*. 2005;92(12):2140–7.
- Uchio EM, Linehan WM, Figg WD, Walther MM. A phase I study of intravesical suramin for the treatment of superficial transitional cell carcinoma of the bladder. *J Urol*. 2003;169(1):357–60.
- Gao X, Au JL, Badalament RA, Wientjes MG. Bladder tissue uptake of mitomycin C during intravesical therapy is linear with drug concentration in urine. *Clin Cancer Res*. 1998;4(1):139–43.
- Wientjes MG, Dalton JT, Badalament RA, Dasani BM, Drago JR, Au JL. A method to study drug concentration-depth profiles in tissues: mitomycin C in dog bladder wall. *Pharm Res*. 1991;8(2):168–73.
- Wientjes MG, Dalton JT, Badalament RA, Drago JR, Au JL. Bladder wall penetration of intravesical mitomycin C in dogs. *Cancer Res*. 1991;51(16):4347–54.
- Dalton JT, Wientjes MG, Badalament RA, Drago JR, Au JL. Pharmacokinetics of intravesical mitomycin C in superficial bladder cancer patients. *Cancer Res*. 1991;51(19):5144–52.
- Song D, Wientjes MG, Au JL. Bladder tissue pharmacokinetics of intravesical taxol. *Cancer Chemother Pharmacol*. 1997;40(4):285–92.
- Ogden A, Wientjes MG, Au JL. Suramin as a chemosensitizer: oral pharmacokinetics in rats. *Pharm Res*. 2004;21(11):2058–63.
- Song D, Wientjes MG, Gan Y, Au JL. Bladder tissue pharmacokinetics and antitumor effect of intravesical 5-fluorouridine. *Clin Cancer Res*. 1997;3(6):901–9.
- Stein CA, Khan TM, Khaled Z, Tonkinson JL. Cell surface binding and cellular internalization properties of suramin, a novel antineoplastic agent. *Clin Cancer Res*. 1995;1(5):509–17.
- Au JL, Lu Z, Yeh TK, Lyness G, Chen L, Xin Y, *et al*. Rapid release paclitaxel nanoparticles for intravesical therapy in dogs with spontaneous bladder cancer. *J Urol*. 2009;181(4):635.
- Vansterkenburg EL, Wilting J, Janssen LH. Influence of pH on the binding of suramin to human serum albumin. *Biochem Pharmacol*. 1989;38(18):3029–35.
- Collins JM, Klecker Jr RW, Yarchoan R, Lane HC, Fauci AS, Redfield RR, *et al*. Clinical pharmacokinetics of suramin in patients with HTLV-III/LAV infection. *J Clin Pharmacol*. 1986;26(1):22–6.
- Levitt DG. The pharmacokinetics of the interstitial space in humans. *BMC Clin Pharmacol*. 2003;3:3.
- Walther MM, Trahan EE, Cooper M, Venzon D, Linehan WM. Suramin inhibits proliferation and DNA synthesis in transitional carcinoma cell lines. *J Urol*. 1994;152(5 Pt 1):1599–602.
- McNally WP, DeHart PD, Lathia C, Whitfield LR. Distribution of [^{14}C]suramin in tissues of male rats following a single intravenous dose. *Life Sci*. 2000;67:1847–57.