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M. Guillaume Wientjes · Robert A. Badalament
Jessie L.-S. Au

Penetration of intravesical doxorubicin in human bladders

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Abstract The bladder wall penetration kinetics of intravesical doxorubicin were examined in radical cystectomy patients, to provide insight into drug concentrations at target tumor sites. The dosing solution (40 mg/20 ml) was instilled just prior to the start of surgery and maintained for 60–115 min until just prior to bladder excision. The data showed considerable inter-patient variability in the peak plasma concentration (24-fold), urine concentration (7-fold), and tissue concentration (28-fold). The urine concentration at the time of tissue harvest was about 17% of the concentration in the dosing solution. This was due to the dilution by post-catheterization residual urine and urine produced during treatment. The doxorubicin concentration dropped by 32-fold across the urothelium, and declined semi-logarithmically with respect to depth in the capillary-perfused tissues beneath the urothelium with a 50% decrease over about 500 μm. In three of six patients from whom tumor tissue was obtained, the doxorubicin concentration was higher than the adjacent non-tumor-bearing tissues of comparable tissue depth, whereas the reverse was seen in the remaining three tumors. The plasma concentrations were 0.02, 0.03, 0.05, 0.27, and 0.69% of the concentrations in the tumors, urothelium, lamina propria, superficial and deep muscle layers, respectively. These data indicate: (a) a considerable intra- and inter-patient variability in bladder tissue concentrations, in part due to the variability in the urine concentration; (b) the urothelium is an effective barrier to doxorubicin penetration; and (c) a targeting advantage of intravesical therapy for the treatment of superficial bladder cancer yielding superficial bladder tissue concentrations at least 2000-fold higher than in the systemic circulation. A comparison of the data of doxorubicin with our previously published data on mitomycin C shows similar bladder tissue pharmacokinetics for the two drugs, suggesting that there is no pharmacokinetic preference for either drug.

Key words Doxorubicin · Bladder cancer

M.G. Wientjes (✉) · R.A. Badalament
College of Pharmacy and Division of Pharmaceutics and Urology, The Ohio State University, Columbus, Ohio 43210, USA

J.L.-S. Au
College of Pharmacy, Division of Pharmaceutics, The Ohio State University, 500 West 12th Avenue, Columbus, OH 43210, USA
Tel. (614) 292-4244

Introduction

Intravesical chemotherapy is commonly used in combination with transurethral resection for treatment and prophylaxis of superficial bladder cancers [1]. Compared to patients treated by resection alone, those receiving adjuvant intravesical chemotherapy of mitomycin C, doxorubicin or thiotepa have a reduced tumor recurrence rate [1–4]. The response rates to these three drugs are highly variable, ranging from 2% to 43% for mitomycin C, 17% to 54% for doxorubicin, and 8% to 26% for thiotepa [2]. Clinical studies have shown that the response rate to intravesical chemotherapy is determined by the location and the pathology of the tumors. For example, low stage, low grade tumors are more likely to show a favorable response than muscle invading, high grade tumors [5].

Our laboratories have been studying the pharmacokinetics and pharmacodynamics of agents used in intravesical chemotherapy of superficial bladder cancer. These studies are designed to elucidate if the variable patient response is due to variable pharmacokinetics and drug concentration at the tumor sites, and/or variable response of individual patient tumors to the drug. The overall goal is to use the pharmacokinetic and pharmacodynamic data to devise treatment strategies to improve the therapeutic efficacy. The completed studies on mitomycin C include the plasma and urine pharmacokinetics in superficial

bladder cancer patients treated with intravesical therapy [6], the bladder tissue pharmacokinetics in radical cystectomy patients and in dogs [7, 8], and the response of patient tumors to the drug [9]. A comparison of the tissue pharmacokinetic data and the pharmacodynamic data indicates that the variable patient response to intravesical mitomycin C therapy is likely due to the 10 to 20-fold intra- (between treatments) and inter-patient variability in the target site pharmacokinetics, and the 120-fold variability in the tumor response of individual patient tumors to the drug. The data further suggest that the lower efficacy of intravesical mitomycin C therapy in the high stage and high grade tumors compared to the superficial tumors is likely due to the combined effects of low drug exposure at the deep tissue sites and the relatively lower tumor sensitivity to the drug [8, 9]. These results were used to design an optimized treatment regimen that is projected to have a 20% higher efficacy [10].

We have completed the study on plasma and urine pharmacokinetics of doxorubicin in superficial bladder cancer patients [11]. The present investigation was to examine in patients the penetration of doxorubicin into bladder tissues, in order to establish the tissue pharmacokinetics of doxorubicin and the extent of increase of tissue concentrations due to the intravesical administration route. The present study was done in patients who had muscle invading disease and required radical cystectomy.

Materials and methods

Chemicals and instruments

Epirubicin was a gift from Adria Laboratories (Columbus, Ohio). Doxorubicin was purchased from Cetus (Emeryville, Calif.). High pressure liquid chromatography (HPLC) grade chemicals and solvents were purchased from Fisher Scientific (Fair Lawn, N.J.) and J.T. Baker Chemical (Phillipsburg, N.J.), and Supelclean 1-ml LC-18 solid phase extraction tubes from Supelco (Bellefonte, Pa.). All chemicals were used as received. The HPLC system consisted of a solvent pump (Applied Biosystems, model 400, Foster City, Calif.), a model 712 automated sampler, a model 470 scanning fluorescence detector (Waters, Milford, Mass.), and an integrator (Hewlett-Packard, model 3396A, Avondale, Pa.).

Patient protocol

Patients had histologically confirmed bladder cancer and received radical cystectomy as a part of medical management. Table 1 summarizes the patient characteristics. Immediately prior to surgery, the bladder was emptied and 40 mg doxorubicin in a 20-ml 0.9% NaCl solution was instilled. The bladder was again emptied just prior to surgical excision of the bladder. Urine samples were taken every 30 min for the duration of the instillation, which ranged from 60 to 115 min, and blood samples every 30 min for 2 h. Bladder emptying, drug instillation and urine sampling were via an in-dwelling urethral catheter, and blood samples via an in-dwelling catheter in a peripheral vein. The surgical procedures for ureter ligation, bladder excision and tissue removal were as described previously [12]. One

Table 1 Patient characteristics. Patient 3 had squamous cell carcinoma. All other patients had transitional cell carcinoma. Stage and grade are as defined by Pauli et al. [26]

Patient number	Age years	Stage	Grade	Interval between transurethral resection and cystectomy (days)
1[a]	62	P3bN0M0	3	330
2	74	P3aN0M0	3	5
3	77	P3bN0M0	3	11
4	73	P1N0M0	3	45
5	62	P3bN0M0	2–3	9
6	62	P3bN0M0	3	18
7	41	P3bN1M0	3	43
8	54	P2N1M0	3	17
9	74	P4N*x*M0	3	84
10	73	P3aN0M0	3	13

[a]Treated by radiation 270 days before surgery

to two transmural portions of tissue without apparent lesions were flash-frozen. The urothelial surface area of each specimen was approximately 3 cm^2. The time between surgical removal of the bladder and freezing of the bladder tissue specimens was less than 6 min. Tumor samples were collected whenever possible to compare the drug concentrations in tumor and normal tissues. Tumor specimens were obtained after opening of the bladder from areas that appeared non-necrotic. Tissue, plasma and urine samples were stored frozen at $-70°C$ until analysis.

Tissue processing

The frozen bladder tissues were sectioned as previously described [12]. Briefly, the surface of tissues were trimmed with a scalpel to remove residual drug solution. Sections (40 μm) parallel to the urothelium were obtained by cryotome sectioning. When appropriate, multiple samples were pooled for analysis.

Sample analysis

Doxorubicin concentrations in plasma, urine, and bladder tissue samples were analyzed by HPLC. The HPLC method and the procedures for the clean-up of plasma and urine samples were as published earlier [11]. Briefly, plasma samples were processed with solid-phase extraction tubes. Urine samples were diluted to an appropriate concentration range and analyzed without extraction. Tissue sections were homogenized for 2 min after adding the internal standard, epirubicin, and 5 ml of acetone. The homogenized samples were centrifuged for 20 min, and the organic layer was collected, evaporated to near-dryness and reconstituted with mobile phase before HPLC analysis.

Data analysis

As discussed previously, drug transport in the bladder wall can be represented mathematically as two processes [7, 8]. The first is passive diffusion across the urothelium, which functions as an absorption barrier. The second is a combination of drug diffusion and drug removal by the perfusing capillaries in the lamina propria and the muscle layers, as described by the distributed model. The

doxorubicin concentration decline across the urothelium and in the capillary-perfused tissues was analyzed by Eqs. 1 and 2, respectively:

$$C_x = C_u - \frac{C_u - C_{uro}}{x_{uro}} \cdot x \qquad \text{Eq. 1}$$

$$C_x = (C_{uro} - C_b) \cdot e^{-\frac{0.693}{w_{1/2}} \cdot (x - x_{uro})} + C_b \qquad \text{Eq. 2}$$

where C_x is the concentration at a tissue depth x, C_{uro} is the concentration at the junction of urothelium and lamina propria, half-width ($w_{1/2}$) is the tissue thickness over which the concentration declines by 50%, and x_{uro} is the thickness of the urothelium, which is about 200 μm in human bladders [8]. C_b is the drug concentration in the tissue in equilibrium with the perfusing blood. At tissue depths much greater than $w_{1/2}$, the equation is reduced to $C_x = C_b$. Hence, the drug concentrations in deep tissues reach a plateau value equalling C_b. Because the drug readily partitions into the capillaries, the drug concentration in the vesical vein leaving the bladder is significantly higher than the incoming blood concentration.

The doxorubicin urine concentration-time profiles in patients were analyzed using Eq. 3, as previously described [11]:

$$C_u = \frac{\text{Dose}}{V_u} \quad \text{where} \quad V_u = V_o + k_o \cdot t + V_{res} \qquad \text{Eq. 3}$$

where C_u is the urine concentration of doxorubicin at time t during instillation, V_o is the volume of the doxorubicin dosing solution, k_o is the zero order rate constant describing urine production, and V_{res} is the volume of post-catheterization residual urine at the time of drug instillation. Because our previous study showed an insignificant doxorubicin removal from the bladder cavity by absorption into the systemic circulation or metabolism [11], these processes were not represented in Eq. 3. For all ten patient bladders, concentration data were available for a depth of at least 5520 μm. The average tissue concentration was calculated by dividing the area under the tissue concentration-depth profile over the tissue depth from 200 to 5520 μm.

Computer fitting was done using the NLIN procedure (SAS Institute, Cary, N.C.). Statistical analysis was by paired or unpaired two-tailed Student's *t*-tests using the SAS TTEST or MEANS procedures. A *P* value of $\leq 5\%$ is considered significant.

Results

Urine and plasma pharmacokinetics

Figures 1A and B show the urine and plasma concentration-time profiles, respectively. Table 2 summarizes the pharmacokinetic parameters. After instillation, the concentration of doxorubicin in urine dropped by 70% from a concentration of about 2200 μg/ml in the dosing solution to about 600 μg/ml at the first sampling time of 30 min. The analysis of the concentration-time profile by Eq. 3 suggests that this was due to the residual post-catheterization urine in the bladder at the time of drug instillation. The volume of the residual urine in eight of the ten patients was significant compared to the volume of the dosing solution (20 ml). The

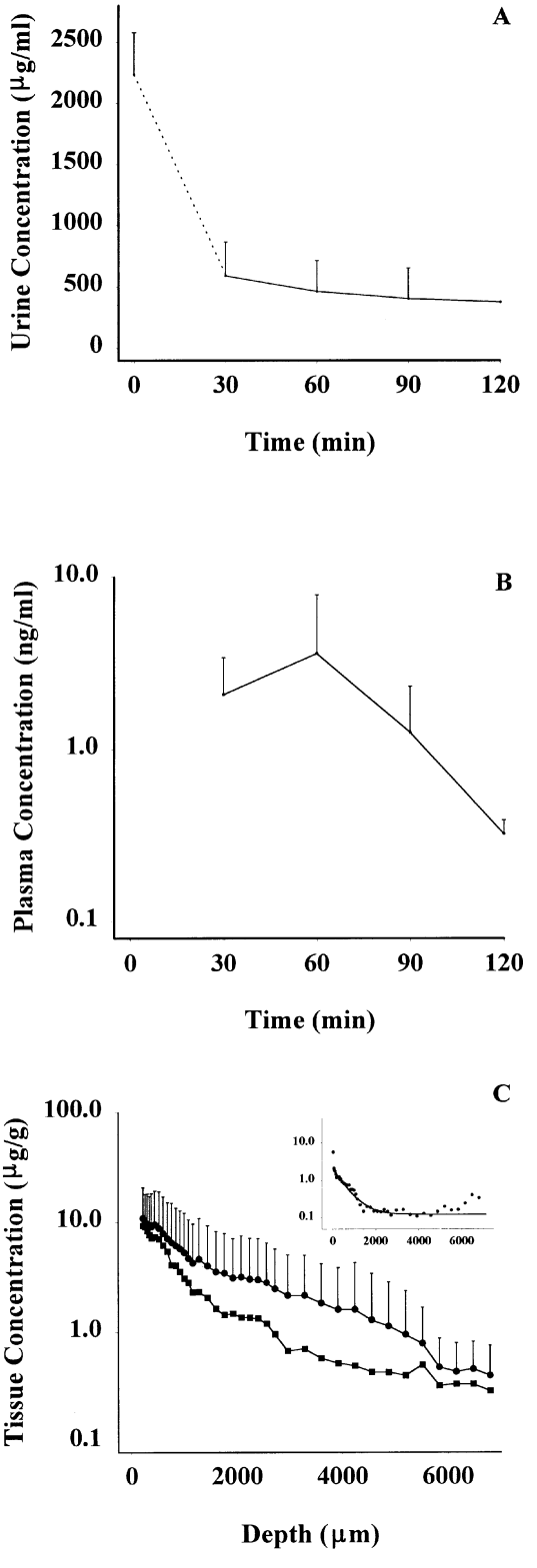

Fig. 1A–C Urine, plasma and tissue pharmacokinetics in patients. Patients received 40 mg doxorubicin in 20 ml physiological saline by intravesical instillation for 60–115 min. **A** Plot of urine concentration-time profile. Data are mean + one SD ($n = 10$ for time points up to 60 min, $n = 3$ at 90 min, and $n = 1$ at 120 min). The concentration at 0 min was the mean concentration of the dosing solution. **B** Plasma concentration-time profile. Data are mean + one SD ($n = 10$). **C** Tissue concentration-time profile. Mean (●) and median (■) are presented. *Bar* is SD The inset shows the profile of patient 9. Note that the urine and tissue concentrations are in μg/ml and the plasma concentrations are in ng/ml

Table 2 Doxorubicin concentrations in bladder tissue, urine and plasma in patients. Patients received intravesical treatment of doxorubicin (40 mg in 20 ml physiological saline) prior to start of surgery. C_{dose} concentration in dosing solution, $C_{u,\,harvest}$ urine concentration at the time of bladder removal, C_{uro} concentration at the junction between urothelium and lamina propria where T_a tumors are located, C_{700} concentration at 700 μm (lamina propria), C_{2000} concentration at 2000 μm (muscularis), $C_{average}$ average tissue concentration, C_b minimum plateau concentration; C_{tumor} concentration in papillary tumor tissue, $C_{p,\,max}$ maximum plasma concentrations, $w_{1/2}$ thickness of the tissue overwhich the concentration declines by one-half, k_o urine production rate, V_{res} residual urine volume. C_b was reached between 2000 and 3000 μm for patients 1, 2, 3, 4, 5, and 9, and between 3500 and 7000 μm for patients 6, 7, 8 and 10

Patient Number	C_{dose} (μg/ml)	$C_{u,\,harvest}$ (μg/ml)	C_{uro} (μg/g)	C_{700} (μg/g)	C_{2000} (μg/g)	$C_{average}$ (μg/g)	C_b (μg/g)	C_{tumor} (μg/g)	$C_{p,\,max}$ (ng/ml)	$W_{1/2}$ (μm)	k_o (ml/min)	V_{res} (ml)
1	2535	275	15.7	6.19	1.16	2.22	0.77	10.5	2.8	342	0.0	168
2	2345	166	9.43	8.90	9.93	6.87	0.63	16.9	2.4	295	2.1	0
3	2826	157	6.56	3.12	0.62	1.27	0.25	24.7	2.8	439	2.1	0
4	1803	402	10.3	6.26	2.05	4.91	0.70	4.1	14.8	637	0.2	60
5	2075	285	3.10	1.78	0.50	1.44	0.16	–[a]	1.6	579	0.0	132
6	2288	726	7.83	4.46	1.12	1.47	0.16	19.1	1.2	600	0.0	30
7	1974	110	5.31	2.37	0.40	0.73	0.15	–[a]	1.2	411	1.5	105
8	2446	769	11.6	7.21	2.28	2.67	0.43	–[a]	5.3	694	0.3	22
9	2334	492	1.41	0.61	0.16	0.27	0.12	–[a]	0.6	356	0.6	37
10	1727	484	38.7	27.5	11.5	11.0	0.73	4.2	5.2	990	0.4	10
Mean	2235	387	11.0	6.84	2.97	3.28	0.41	13.3	3.8	534	0.7	56
SD	± 341	± 232	± 10.6	± 7.71	± 4.15	± 3.37	± 0.27	± 8.4	± 4.2	± 211	± 0.9	± 59
Median	2182	110	8.63	5.33	1.14	1.84	0.34	13.7	2.6	538	0.3	34

[a]Tumor tissue was not available

urine concentration continued to decline at a much slower rate to about 400 μg/ml at 115 min, or when the bladder was excised. This later slow urine concentration decline was due to the dilution by urine produced and collected in the bladder during treatment in all except three patients whose ureters were ligated at the beginning of the surgery.

The doxorubicin concentration in plasma, due to absorption from the bladder, reached a maximum at or near the time of bladder excision and declined after removal of the bladder. The maximum plasma concentration was less than 0.001% of the urine concentration at the time of bladder excision ($C_{u,\,harvest}$).

Bladder tissue concentrations

Figure 1C shows the mean and median bladder tissue concentration-depth profiles. Table 2 summarizes the tissue pharmacokinetic parameters including the drug concentrations at different tissue depths, and the $w_{1/2}$. In nine patients, the median ratio of tissue concentration at the urothelium and lamina propria interface (C_{uro}) compared to the $C_{u,\,harvest}$ was 32, whereas the mean ratio was higher at 42 due to an outlier (patient 9). In nine patients, the drug concentrations in the lamina propria and muscularis declined semi-logarithmically with a $w_{1/2}$ of about 500 μm until a relatively constant level was reached. The doxorubicin concentration in the lamina propria (~700 μm, C_{700}) and the superficial muscle layer (~2000 μm, C_{2000}) were about 60% and 27% of the C_{uro}, respectively. In five patients, the concentration began to level off at between 2000 and 3000 μm (inset, Fig. 1C), whereas in the remaining four patients, the minimal concentration was reached between 3500 and 7000 μm. The drug concentration in the deep muscle layer was about 4% of the C_{uro}. In the remaining patient (patient 2), the doxorubicin concentration stayed at a plateau of about 9 μg/ml from 200 to 2500 μm, then declined with a $w_{1/2}$ of 295 μm to a second plateau of 0.6 μg/ml at 5500 μm.

Concentrations in tumors

Tumor tissues were available from six patients. These were papillary tumors consisting of stalks of tumor tissue growing from a central area and lacking a smooth surface. Hence, the concentration-depth profiles for these tumors could not be determined, and only the average concentration is presented (Table 2). The ratio of mean C_{tumor} to the mean concentration of tissues of comparable depth in the grossly normal region, i.e., C_{uro} or the average of C_{uro} and C_{700}, were higher than 1 (Table 3). However, the differences in concentrations in tumor and grossly normal tissues were not statistically significant ($P > 0.80$).

Inter-subject variability

There was considerable inter-subject variability in the ten patients. The doxorubicin concentration in the dosing solution showed a 50% variation. However the

Table 3 Ratios among urine, plasma, tissue and tumor concentrations. The abbreviations are as described in Table 2

Patient number	$C_{u,\,harvest}$: C_{ure}	C_{ure}: $C_{p,max}$	C_{700}: $C_{p,max}$	C_{2000}: $C_{p,max}$	C_b: $C_{p,max}$	$C_{average}$: $C_{p,max}$	C_{tumor}: $C_{p,max}$	C_{tumor}: C_{uro}	C_{tumor}: average (C_{uro} and C_{700})[a]
1	17.5	5602	2210	413	274	791	3750	0.67	0.96
2	17.6	3929	3708	4138	263	2861	7042	1.79	1.84
3	23.9	2343	1113	222	90.8	455	8821	3.77	5.10
4	39.1	695	423	138	47.1	332	277	0.40	0.50
5	91.9	1939	1112	315	102	899	_[a]	_[b]	_[b]
6	92.8	6522	3719	933	134	1222	15917	2.44	3.11
7	20.7	4424	1976	332	125	611	_[a]	_[b]	_[b]
8	66.2	2191	1361	430	80.3	503	_[a]	_[b]	_[b]
9	350	2343	1012	268	204	442	_[a]	_[b]	_[b]
10	12.5	7444	5286	2211	140	2108	808	0.11	0.13
Mean	42.5[c]	3743	2192	364[d]	145	1022	6102	1.53	1.94
SD	± 32.6	± 2217	± 1556	± 223	± 76.9	± 833	± 5867	± 1.41	± 1.88
Median	31.5	3136	1669	332	129	701	5396	1.23	1.40

[a]The average values of C_{uro} and C_{700} were 11.5, 9.17, 4.84, 8.28, 6.15, 33.1 μg/g for patients 1, 2, 3, 4, 6, and 10, respectively. The mean ± SD of these averages was 12.2 ± 10.5 μg/g
[b]Tumor tissue was not available
[c]The value shown was calculated by excluding the outlier (patient 9). The mean ± SD including patient 9 was 73 ± 102
[d]The value shown was calculated by excluding the outlier (patient 2). The mean ± SD including patient 2 was 940 ± 1280

$C_{u,\,harvest}$, C_{uro}, C_{700}, and C_{2000}, showed a much greater variation of 7-, 27-, 45-, and 72-fold, respectively. With the exclusion of the two outliers (patients 2 and 10), C_{uro}, C_{700}, and C_{2000} showed a still relatively wide range of 11-, 12- and 14-fold. Likewise, the average tissue concentration between tissue depths of 200 and 5520 μm ($C_{average}$) showed a 40-fold range for all ten patients, and an 18-fold range when patients 2 and 10 were excluded. Interestingly, the variation in the minimum plateau tissue concentration (C_b) and the tumor tissue concentration was relatively small, both with a 6-fold range. The peak plasma concentration showed a 25-fold range, and a 9-fold range when the one outlier (patient 4) was excluded.

Ratios among urine, plasma, tumor, and non-tumor-bearing bladder tissue concentrations

Table 3 summarizes the ratios among doxorubicin concentrations in urine, plasma, grossly normal bladder tissue, and bladder tumor tissue. Compared to the systemic peak plasma concentration, the urothelium, lamina propria, superficial muscle, and deep muscle layers (5520 μm) received a ≥3700-, 2100-, 360- and 140-fold higher concentration, respectively (Table 3). The average tissue concentration was 1000-fold higher than the peak plasma concentration.

Discussion

The objectives of the present study on doxorubicin are fourfold: (a) to define the bladder tissue pharmacokinetics of intravesical doxorubicin therapy; (b) to define the inter-patient variability in target site pharmacokinetics; (c) to define the tissue targeting advantage (i.e., the ratio of drug concentration and exposure at the target tumor sites compared to the plasma concentration); and (d) to compare the data of mitomycin C and doxorubicin in order to gain insight on the characteristics of bladder tissue penetration by different drugs. As discussed above and elsewhere [10], these data are critical to elucidate the pharmacokinetic basis of the efficacy of intravesical chemotherapy.

Bladder tissue pharmacokinetics

The 32-fold drop of doxorubicin concentration across the urothelium indicates that the urothelium is an effective barrier to drug absorption. Enhancement of drug delivery to the deep muscle layers may be accomplished by using absorption enhancers. Preliminary data of an ongoing study in our laboratories indicate that the absorption barrier function of the urothelium may be partly overcome by the use of dimethylsulfoxide [13], in agreement with the observation in rats by See and Xia [14].

Previously reported studies have investigated the concentrations of doxorubicin in bladder tumors and biopsy specimens of apparently normal bladder wall tissue [15–18]. These studies were performed in patients treated with intravesical cancer chemotherapy. Transmural tissue specimens, like those used in our studies, were therefore not available, and concentration-depth profiles could not be obtained. One study reported a 10 μg/g doxorubicin concentration in normal mucosa obtained 2 h after a 50-mg dose in 30 ml of

saline [16]. This is similar to the mean urothelial concentration of 11 μg/g in our study. This earlier study [16] did not report the $C_{u,\,harvest}$, hence the concentration gradient across the urothelium could not be evaluated. Two studies reported a 2- to 3-fold higher tumor concentration compared to that of the normal mucosa [16, 18], whereas a third study reported a similar concentration in tumor and normal tissue [15]. One study reported a 40000-fold ratio of urine to plasma concentrations, based on measurement of total radioactivity [15]. This is lower than the 100000-fold difference found in the present study. The bladder tissue concentration for a doxorubicin analog, idarubicin, has been reported; the tumor concentration was approximately 2- to 3-fold higher than that in normal mucosa and the concentration in the muscle layer was approximately 10% of the mucosal concentration [19]. In comparison, data of the present study show substantial variability in the ratio of tumor concentrations to normal mucosal concentrations, with a tendency for tumor concentrations to exceed the normal tissue concentrations. However, it is noted that removal of urine from the tumor tissues, because of their rough edges and "cauliflower" structure, may be less likely to be complete compared to the normal tissues, which had a flat surface. Because the urine concentration was significantly higher than the tissue concentration, contamination of tissue by the residual urine may lead to erroneously high tissue concentrations.

Intersubject variability

The major determinants of the bladder tissue concentration-depth profile are: (a) drug concentration in the urine; (b) drug penetration across the urothelium; (c) drug diffusion in the extracellular space in the bladder tissues; and (d) drug removal by the perfusing blood. Data in Table 2 show a significant inter-subject variability (6- to 72-fold) in the drug concentration in different parts of the bladder wall (i.e., C_{uro}, C_{700}, C_{2000}, C_b, and $C_{average}$). This variability is, in part, a cumulative effect of the variability in urine concentrations, combined with the variabilities in barrier function of the different tissue layers. Extensive variability was also observed in our previous studies with mitomycin C in dog and human bladders [7, 8], and with doxorubicin in dog bladders (unpublished observations). In addition to random variability, our previous studies suggested inflammation, healing of surgical wounds, and overdistention of the bladder wall as possible factors for increased permeability. A large intersubject variability is also encountered in transport through other biological membranes. For example, a greater than ten-fold range in percutaneous absorption was found for taurocholic acid in the thigh skin of three individuals [20].

Elimination of the variability in tissue concentrations may help to reduce the variability in the response of patients to the therapy. Among the multiple determinants, the variability in the urine drug concentration may be the one that is most easily controlled. This may be accomplished through a reduction in the post-catheterization residual urine (e.g., by more completely emptying the bladder prior to drug administration) and a reduction in the urine production rate (e.g., by restricting fluid intake). As discussed above, drug penetration across the urothelium may be enhanced by the use of an absorption enhancer, but this may or may not reduce the intersubject variability. Drug diffusion in the extracellular space and drug partitioning across the capillary membrane are a function of the drug properties, and are not likely to be altered for a given drug. Drug removal by the perfusing blood is a function of the blood flow. The effect of altering blood flow on the tissue concentration-depth profile has not been defined.

Tissue targeting by intravesical therapy

Intravesical chemotherapy is used to treat TIS, Ta, and T1 tumors located in the urothelium and lamina propria. The superficial diseases generally show a better response rate than the muscle-invading diseases such as T2, T3, and T4, located in the superficial and deep muscle layers. Data in Table 3 show a >2000-fold higher drug concentration in the urothelium and lamina propria compared to the systemic plasma concentration. This compares favorably to the tissue-to-plasma ratio of 29-fold for muscle tissue in rabbits [21], and indicates a significant tissue targeting due to the intravesical treatment route for superficial disease. In the high stage, muscle invading tumors that are located in the superficial and deep muscle layers, the targeting advantage is significantly lower; the concentrations in these tissues were only 364- and 145-fold higher than the peak plasma concentration. The substantially different drug concentration in the superficial tissues (urothelium and lamina propria) and in the deep tissues (superficial and deep muscle layers) may contribute to the different response rate among the low and high stage diseases.

Because of the requirement for a substantial amount of tissue for concentration analysis, the present study was performed in patients that required radical cystectomy. These patients, in general, have more extensive disease than patients that are treated with intravesical chemotherapy for superficial bladder tumor. We recently reported the plasma and urine pharmacokinetics of doxorubicin in the latter patient group [11]. A comparison of the results in the previous and present studies shows similar values for the ratio of maximal plasma concentration to urine concentration and similar post-catheterization residual urine volumes in these two patient groups. However, compared to the patients with superficial disease, cystectomy patients showed higher maximal plasma concentrations (3.8 ± 4.2 ng/ml, $n = 10$ versus 0.4 ± 1.0 ng/ml, $n = 23$) and

lower urine production rates (0.7 ± 0.9 ml/min, $n = 10$ versus 2.5 ± 1.3 ml/min, $n = 23$). The lower urine production in the cystectomy patients was apparently due to early ligation of the ureters during surgery, whereas the higher plasma concentrations may be due to the more advanced disease state and/or surgical manipulation of the bladder. While a higher plasma concentration may suggest a higher drug penetration into the bladder wall, data of the present study did not show a statistically significant correlation between the plasma concentration and tissue concentration ($P > 0.25$, $r = 0.4$). This may be due to confounding factors, such as unequal duration of instillation.

Comparison of mitomycin C and doxorubicin data

A comparison of our previously reported bladder tissue pharmacokinetic data on mitomycin C [8] and the present data on doxorubicin indicates a striking similarity in the human bladder tissue penetration characteristics by the two drugs. The absorption of both drugs was limited by their penetration across the urothelium with a 30-fold concentration gradient; only a small fraction of the dose, i.e., $<2\%$ was present in the tissue. The tissue pharmacokinetics of both drugs were described by the distributed model, which indicates that the drug transport in the tissue is by passive diffusion and by removal via the perfusing capillaries, with a 50% decrease in drug concentration over a tissue depth of about 500 μm. The ratios of tissue concentration (C_{tumor}, C_{uro}, C_{700}, C_{2000}, C_b, and $C_{average}$) to the peak plasma concentration ranged from 145 to 6000 for doxorubicin. For mitomycin C, these ratios were substantially lower at between 5- and 840-fold. The higher tissue targeting advantage of doxorubicin is not due to a more rapid total body clearance, because these drugs have similar clearances, i.e., 6 and 4 ml/kg per min for mitomycin C and doxorubicin, respectively [22, 23]. The tissue binding of doxorubicin is 10-to 500-fold higher than its plasma protein binding [23]. The analytical methodology used in the present study analyzes the total concentration, i.e., free and reversibly bound doxorubicin. It is possible that the higher tissue-to-plasma concentration ratio of doxorubicin compared to mitomycin C is due to the preferential tissue binding of doxorubicin. For both drugs, the peak plasma concentration after intravesical administration is well below the threshold toxic level, i.e., 400 ng/ml for mitomycin C [24] and 500 ng/ml for doxorubicin [25]. Hence, while the higher tissue targeting advantage of intravesical doxorubicin therapy represents a theoretical advantage over mitomycin C, there is no preference based on the bladder tissue pharmacokinetic data.

In summary, the present study established the bladder tissue pharmacokinetics of doxorubicin in patients. The data indicate an extensive tissue targeting advantage of intravesical instillation, and a 6- to 72-fold inter-subject variability in the target tissue concentration. The similarity between the bladder tissue penetration of doxorubicin and mitomycin C suggests that there is no pharmacokinetic rationale to select one drug versus the other. Comparative pharmacodynamic data, i.e., the drug concentration needed to produce an antitumor effect, on the two drugs are needed to reach conclusions on their relative efficacy. We further hypothesize that minimizing the post-catheterization residual urine volume and the urine production rate, and enhancing the drug penetration across the urothelium may partly overcome the inter-subject variability, and will improve the therapeutic efficacy. These questions are being addressed in ongoing studies in our laboratories.

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