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Bladder tissue pharmacokinetics of intravesical taxol

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Abstract Our previous studies have suggested that the ineffectiveness of intravesical mitomycin C or doxorubicin therapy against muscle-invading bladder cancer is in part because of the inability of these drugs to penetrate the urothelium (the urothelial drug concentration is $< 5\%$ of the concentration in urine). The goal of the present study was to identify agents that are efficiently absorbed across the urothelium. To evaluate the potential use of taxol in intravesical therapy for bladder cancer, we examined the bladder tissue and systemic plasma pharmacokinetics of intravesical taxol in dogs. Animals (~8 kg body weight) were given an instillation of taxol at 500 μg in 20 ml water. At 120 min postinstillation, the bladder was emptied and excised, and about 85% of the dose was recovered in the urine. The taxol concentration in the urothelium was about 50% of the concentration in the urine, the concentrations then declined logarithmically in the underlying capillary-perfused tissues. The average tissue concentration (~2 μg/g) was two to three times the reported plasma concentration of 0.75 μg/ml in patients following intravenous infusion of the >100-fold higher dose of 250 mg/m^2. The steady-state plasma concentration was $< 0.02\%$ of the average tissue concentration, and was $< 0.05\%$ of the maximally tolerated plasma concentration in patients. The octanol:water partitioning coefficients of taxol, doxorubicin, and mitomycin were >99, 0.52, and 0.41, which parallels the rank order of the partitioning across urothelium, i.e. taxol (~50%) $\gg$ doxorubicin $\approx$ mitomycin C (~3%). In summary, the partitioning of taxol across the urothelium was more favorable than the partitioning of mitomycin C and doxorubicin, and the systemic concentration of taxol resulting from intravesical treatment was insignificant in spite of the extensive absorption into the bladder. We conclude that intravesical delivery of taxol provides a significant bladder tissue targeting advantage, and that taxol represents a viable candidate drug for intravesical bladder cancer therapy.

Key words Bladder cancer · Intravesical chemotherapy · Taxol · Urothelial drug penetration

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Introduction

About 60% of the 50 000 annual new cases of superficial bladder cancer will recur after complete endoscopic removal of the initial tumor and about 10–15% of the patients will subsequently develop muscle-invading tumors [4, 18]. Intravesical chemotherapy is given after transurethral tumor resection to reduce the recurrence of superficial bladder cancer. Intravesical chemotherapy, as a regional therapy, provides the pharmacokinetic advantage of selectively delivering drugs in high concentration to the tumor-bearing bladder while minimizing the systemic exposure. The three DNA-reactive agents, thiotepa, doxorubicin and mitomycin C, are the most commonly used drugs [22, 27].

Muscle-invading bladder cancer is managed by radical cystectomy and systemic chemotherapy [10, 19]. Intravesical chemotherapy is not used because the chemotherapeutic agents effective against superficial disease have limited activity against invasive disease. Investigations have been carried out in our laboratory of the pharmacologic basis for the ineffectiveness of intravesical chemotherapy against tumors located in the

muscle layers. Our results suggest that the ineffectiveness is in part a consequence of the inability of the currently used DNA-reactive agents to penetrate deep muscle layers and in part of the low sensitivity of the more aggressive tumors to these drugs [2, 8, 23, 24, 35]. Logical approaches to develop effective intravesical chemotherapy for muscle-invading disease are to identify drugs that are more effective than the currently used alkylators against the invasive tumors, and drugs that readily penetrate across the urothelium and into the deep tissues. The present study explored the latter approach. Taxol was used as the model drug because of its significant antitumor activity and its favorable physicochemical properties (see below).

Taxol is a natural product isolated from the bark of the Pacific yew tree, *Taxus brevifolia* [1, 16, 20, 21]. It is active against refractory ovarian carcinoma, breast cancer, non-small-cell bronchial carcinoma, and squamous cell carcinoma of the head and neck [20, 21]. Taxol has higher activity than other antimicrotubule compounds, such as vinblastine, against human bladder cancer cells [17]. A phase II study has shown that a 24-h systemic infusion of taxol (250 mg/m^2), given weekly for 3 weeks and in conjunction with 5 μg/kg granulocyte colony stimulating factor, is active against previously untreated locally advanced and/or metastatic bladder cancer [19]. An objective response rate of 42% and a complete response rate of 27% were observed with taxol, which is higher than the response rate of about 30% for other currently used single agents such as cisplatin and methotrexate [19].

In addition to its activity against bladder cancer, taxol has physicochemical properties that differ from agents commonly used for intravesical therapy; its molecular weight of 853 Da is severalfold higher and it is highly lipophilic. These properties may improve the transport of taxol across the urothelium which is the major barrier to drug absorption.

The present study evaluated the penetration of taxol into bladder tissue and absorption into the systemic circulation after intravesical administration. This study was performed in dogs because our previous studies have shown that the bladder tissue pharmacokinetics of intravesical mitomycin C in dogs are similar to those in patients [33, 35].

Materials and methods

Chemicals and equipment

Taxol was a gift from Bristol-Myers Squibb Co. (Wallingford, Ct.), and cephalomannine and epitaxol from the National Cancer Institute (Bethesda, Md.). Agents used for anesthesia and euthanasia were of USP grade and chemicals were of high-pressure liquid chromatographic (HPLC) grade, and were purchased from Fisher Scientific Co. (Fair Lawn, N.J.). The competitive inhibition enzyme immunoassay (CIEIA) kit for taxanes was obtained from Hawaii Biotechnology Group (Aiea, Hawaii). HPLC analysis showed that taxol, cephalomannine, and epitaxol were > 99% pure. All reagents were used as received.

The column-switching HPLC system consisted of a Consta Metric IIIG metering pump (LDC/Milton Roy Co., Riviera Beach, Fl.), a Spectroflow 400 solvent delivery system (Applied Biosystems, Foster City, Calif.), an AS-4000 Intelligent Autosampler with the thermoelectric heating/cooling accessory (Hitachi Instruments, Naperville, Ill.), an electrically actuated multifunctional ten-port HPLC valve (Valco Instruments Co., Houston, Tx.), a model 680 automated gradient controller (Waters Milford, Mass. Associates), and an HP 3966A integrator (Hewlett Packard, Wilmington, De.).

A model EL 340 automated microplate reader (Bio-tek Instruments, Winooski, Vt.) was used for monitoring the color development of the CIEIA assay at a sample wavelength of 405 nm and a reference wavelength of 720 nm.

Animal protocol

Male or female beagle dogs (Hazelton Research Products, Kalamazoo, Mich.) weighing 8.4 ± 2.6 kg (n = 5) were used. Animals were fasted overnight, and allowed access to water. The surgical procedures were as described elsewhere [32, 33]. In brief, a jugular vein was catheterized for the collection of systemic blood samples and a cephalic vein for the administration of anesthetics. A urethral catheter was inserted for the collection of urine samples and the administration of drug solutions. After emptying the bladder through the urethral catheter, an intravesical dose of taxel (500 μg per 20 ml water) was instilled. Serial systemic venous blood and urine samples were taken during the 120-min treatment period. At about 5 min before the surgery or about 115 min after drug treatment, the dog was anesthetized. At 120 min, urine was collected through the urethral catheter and the bladder was removed. The bladder tissue was cut into three sections, (left and right lateral sides, and dome). The tissue sections were snap-frozen in liquid nitrogen, as previously described [32, 33].

Tissue extraction

Frozen transurethral bladder wall tissue samples were cut in parallel to the urothelial surface into 40-μm slices using a cryotome (Carl Zeiss, Thornwood, N.Y.), as previously described [32, 33]. The urothelium layer (first one or two slices) contaminated with urine and blood were discarded. The first ten samples contained one slice each. The next ten samples contained two slices each, and the remaining samples contained four slices each. After weighing, the frozen tissue samples were spiked with 50 μl of the internal standard (cephalomannine, 8.7 μg/ml) and homogenized in 4 ml ethyl acetate using a rotor-stator type homogenizer. The homogenizer probe was washed with 4 ml ethyl acetate to recover residual adhering tissues. The two ethyl acetate fractions were combined and centrifuged at 2000 g for 5 min. The supernatant was transferred and evaporated to dryness under vacuum (SpeedVac, Forma Scientific, Marietta, Ohio). The residue was reconstituted in 100 μl 30% acetonitrile in water, vortexed, and sonicated for 2–3 min, and 15–50 μl was injected into the HPLC system.

HPLC analysis

The HPLC assay analyzed for unchanged taxol and was the preferred method. Tissue samples were analyzed by our previously reported HPLC assay using a column-switching method [28]. Cephalomannine was used as the internal standard. In brief, the HPLC stationary phase consisted of a clean-up column (NovaPak C_8, 75 × 3.9 mm ID, 4 μm particle size, Waters Associates, Milford, Mass.) and an analytical column (Bakerbond octadecyl, 250 × 4.6 mm ID, 5 μm particle size, J.T. Baker, Phillipsburg, N.J.). Samples were injected onto the clean-up column and eluted with the clean-up mobile phase (37.5% acetonitrile in water) at 1 ml/min. Concurrently, the analytical mobile phase (49% acetonitrile) was directed through the analytical column at a flow rate of 1.2 ml/min. The fraction from 8 to 15 min containing taxol and

cephalomannine was transferred from the clean-up column onto the analytical column. The limit of sensitivity for taxol was 1 ng per injection. Urine samples were diluted 11-fold in 45% acetonitrile, spiked with cephalomannine and analyzed by direct injection onto a μBondaPak C_{18} column (3.9 × 300 mm, 10 μm) with a mobile phase of 49% acetonitrile in water at a flow rate of 1.2 ml/min. Taxol has been shown to undergo a reversible epimerization reaction under cell culture conditions [38]. Under the HPLC conditions, epitaxol eluted at 24 min after the taxol peak. Detection of taxol, cephalomannine and epitaxol was at 229 nm.

CIEIA

The CIEIA measures all taxanes but does not distinguish taxol from some of its metabolites. This assay was used to analyze plasma samples where the taxol concentrations were below the detection limit of the HPLC assay. The CIEIA employed a 7-succinyltaxol-bovine serum albumin solid phase coating antigen, a high-titer antitaxane rabbit polyclonal antibody, an alkaline phosphatase conjugated goat antirabbit IgG, and *p*-nitrophenylphosphate as the enzyme substrate. The commercially available CIEIA kit quantifies taxol by its competitive inhibition of the binding of antibody to antigen, and has a detection limit of 0.5 ng/ml for taxol and cephalomannine, and 1 μg/ml for baccatin III and 10-deacetylbaccatin III [11]. The CIEIA required a fourfold dilution of plasma samples to achieve maximum assay sensitivity, thus reducing the actual sensitivity for plasma samples to 2 ng/ml. To improve the assay sensitivity, taxol was extracted from plasma using liquid – liquid extraction which removed plasma proteins that may have interfered with the assay. Plasma samples (0.5 ml) were extracted with 10 ml ethyl acetate, which has been shown to give a >80% recovery of taxol from plasma [28]. The organic layer was transferred and evaporated to dryness under a gentle stream of nitrogen. The residue was reconstituted in 200 μl 0.15 *M* phosphate-buffered saline containing 0.25% bovine serum albumin, 0.05% Tween 20 and 0.02% sodium azide. A 50-μl aliquot of the reconstituted sample was analyzed by the CIEIA. The modification in the CIEIA procedures increased the sensitivity by about tenfold. Standard curves encompassing a drug concentration range of 0.094 to 6 ng/ml were prepared daily and analyzed simultaneously with samples. The standard curves were sigmoidal in shape. Concentrations between 0.2 and 2 ng/ml were within the nearly linear range and higher concentrations corresponded to the plateau range. Hence analysis of higher concentrations required prior sample dilution.

Tissue pharmacokinetic models

For modeling of drug penetration, the bladder wall can be divided into two distinct layers. The covering urothelium is not perfused whereas the underlying connective and muscular layers are perfused by capillaries. Drug penetration through the urothelium is assumed to be via transcellular passive diffusion. In the capillary perfused tissues, the drug is transported by passive diffusion and removed by the capillary flow. We have previously shown that bladder tissue pharmacokinetics of a drug are described by a distributed model [32, 33, 35, 36], as follows:

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

where

$$w_{1/2} = 0.693\sqrt{\frac{D}{pa}}$$

where x is the depth into the tissue layer, C_x is the drug concentration at depth x, C_{uro} is the drug concentration underneath the urothelium, C_b is the drug concentration in the deep muscle layer, which is in equilibrium with capillary plasma drug concentration, half-width ($w_{1/2}$) is the depth over which the ($C_x - C_b$) declines by one-half, p is the capillary permeability, a is the capillary surface area per unit tissue volume, and D is the diffusion coefficient in the capillary perfused tissue layer.

Urine pharmacokinetic model

Urine concentration-time profiles were analyzed using eq. 2, which has been used successfully to describe the urine pharmacokinetics during intravesical treatment [6, 8, 33, 35, 36]:

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

where

$$V_u = V_0 + k_0 t + V_{res} \qquad \text{Eq. 2}$$

where C_u is the urine concentration at time t, V_u is the volume of the urine at time t, $(k_a + k_d)$ is a hybridized first order rate constant describing drug absorption and degradation, V_0 is the volume of dosing solution, k_0 is the zero order urine production rate, V_{res} is the residual urine volume present in the bladder at the time of instillation. The value of k_0 was estimated by the difference in the volumes of the dosing solution and the recovered urine.

Estimation of systemic bioavailability and mass balance

The total body clearance of taxol in dogs is not known. The clearance of taxol is 2.5 to 39.5 ml/min per kg in mice [29] and 11.7 ml/min per kg in humans [20], and the V_{dss} in humans is 2.1 l/kg [20]. These data indicate a relatively narrow range of pharmacokinetic parameters for taxol among different species. We therefore used the clearance and V_{dss} in humans to estimate the systemic bioavailability of the intravesical taxol dose in dogs. The amount of taxol in the body at the end of the experiment was calculated as the product of (plasma taxol concentration at 120 min) and (volume of distribution, V_{dss}), and the amount of taxol cleared from the body during the 2-h treatment was calculated as the product of (taxel clearance and area under the plasma concentration time profile from 0 to 120 min, $AUC_{0-120\ min}$). The $AUC_{0-120\ min}$ was calculated using the trapezoidal rule.

The mass balance was estimated by comparing the dose with the total amount of taxol recovered in the 120-min urine sample, bladder tissue, and systemic circulation, and the fraction converted to epitaxol.

Determination of octanol:water partition coefficient

The partition coefficient of taxol was determined and compared with those of mitomycin C and doxorubicin. Aqueous drug solution (1 ml, 5 μg/ml) was equilibrated with 1 ml water-saturated 1-octanol in 15-ml polypropylene tubes overnight at room temperature. Drug concentration in the aqueous phase was determined. Taxol was measured by HPLC as described above. Mitomycin C and doxorubicin were measured by our previously published HPLC assays [6, 7].

Computer simulation of tissue concentration-depth profiles

Equation 1 states that the tissue concentration-depth profile of a drug is a function of its tissue pharmacokinetic parameters, i.e. C_{uro}, C_b, and $w_{1/2}$. Simulations were performed using these parameters for taxol as defined by the present study, and for mitomycin C and doxorubicin as previously reported [32, 33, 35, 36]. Tissue concentrations were expressed as a function of the C_u. In brief, the C_{uro}:C_u and C_b:C_u ratios were 0.48 and 0.06 for taxol, 0.03 and 0.003 for mitomycin C, and 0.02 and 0.0012 for doxorubicin; and their respective $w_{1/2}$ values were 381, 484, and 534 μm. The theoretical basis for the tissue pharmacokinetic models and the experimental procedures for computer simulations

have been detailed previously [34]. The simulations were done using an IBM-compatible Intel pentium-60 microprocessor-equipped personal computer (Gateway, N. Sioux City, S.D.). Simulations used numerical integration, over 5-min discrete time intervals, programmed in SAS basic language.

Data analysis

Nonlinear least squares regression (NLIN, SAS) was used to analyze the data. Differences between groups were determined with Student's *t*-tests.

Results

Urine concentration-time profiles

Taxol concentrations in urine were measured by an HPLC assay specific for taxol, whereas the concentrations in plasma were measured by immunoassay which measures all taxanes. Therefore the urine data represent unchanged taxol, whereas the plasma data may include taxol metabolites.

Taxol concentrations in urine declined with time during the 120-min treatment (Fig. 1). Table 1 shows the urine pharmacokinetic parameters. The concentration at zero time was the concentration in the dosing solution. The first sample was obtained at 5 min. The 13% concentration decline in the first 5 min was mainly caused by the dilution of the 20-ml dosing solution by the residual urine (~3 ml). The decline in the subsequent 115 min was slower, i.e. 25%, and was caused by further dilution by urine produced during treatment and drug removal by absorption and/or degradation.

The HPLC peak corresponding to epitaxol, although visible, was too small to be quantified. A comparison of the peak heights of the taxol and epitaxol peaks suggests that the epitaxol concentration was $<1\%$ of the taxol concentration. This indicates that epimerization was insignificant, consistent with previous in vivo data [38].

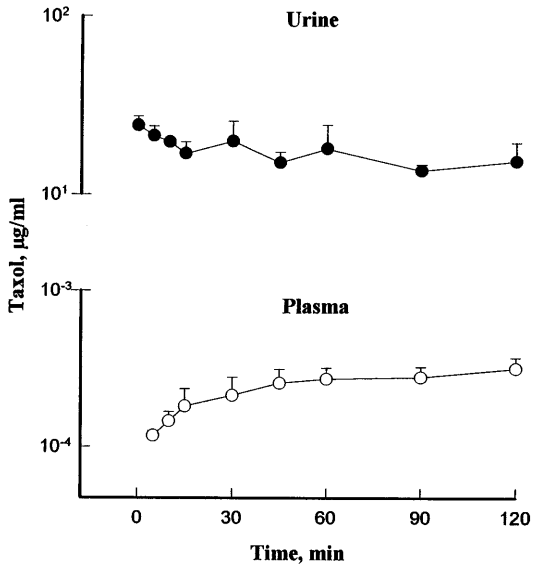

Fig. 1 Concentration of taxol in urine and plasma during intravesical instillation for 120 min. For urine concentrations, the data point at zero time is the concentration in the dosing solution; the later data points represent the concentrations in urine samples obtained serially from the bladder. Values are means + SD ($n = 5$). Note the difference in the scales for urine (µg/ml) and plasma samples (ng/ml). (● DMSO group, ○ water group)

Plasma concentration-time profiles

The plasma concentrations of taxol increased with time, reaching a plateau value of 0.3 ng/ml at 30 min (Fig. 1). The plasma concentrations were several orders of magnitude lower than the concentrations in urine and in bladder tissues (see below). The estimated systemic bioavailability was $<2\%$ (Table 2).

Tissue concentration-depth profiles

Figure 2 shows the taxol concentration in bladder tissue as a function of depth from the urothelium layer, at the end of the 120-min treatment. Table 3 shows the tissue pharmacokinetic parameters. The drug concentration in the urothelium was about 50% of the urine concentration. This indicates that taxol readily partitioned across the urothelium. The concentration of taxol declined logarithmically as it penetrated the capillary-perfused tissues including the lamina propria, and superficial and deep muscularis, and reached a minimum plateau level of about 1 µg/g at 2000 µm depth. The distance for the drug concentration to decrease by 50% was 380 µm.

Mass balance

Table 2 shows the mass balance of the administered dose. About 86% of the dose was recovered in the 120-min urine sample, 1.2% in bladder tissues, 1.8% in plasma, and $<1\%$ as epitaxol. Therefore approximately 90% of the dose was accounted for. This extent of mass balance of an intravesical dose is comparable to our

Table 1 Urine pharmacokinetics of taxol. Five dogs were given an intravesical dose of 500 µg of taxol in 20 ml water. The urine concentration-time profiles were analyzed using eq. 2

	C_u (µg/ml)	Dose recovered in urine (%)	k_0 (µl/min)	V_{res} (ml)	$k_a + k_d$ ($min^{-1} \times 10^{-3}$)
Range	11.6–19.9	78–93	41–108	1.9–5.0	0.63–2.0
Median	14.0	85	67	2.1	1.9
Mean ± SD	15.4 ± 4.2	86 ± 6.2	68 ± 26	3.0 ± 1.7	1.5 ± 0.76

Table 2 Bladder tissue pharmacokinetics of taxol. Five dogs received an intravesical dose of 500 μg taxol in 20 ml water. Bladders were removed after the 120-min treatment and each bladder was divided into dome, and left and right lateral sections and two to three sections were analyzed for drug concentrations versus depth profiles. Data represent the mean of 13 tissues. Bladder tissue pharmacokinetic parameters of taxol were obtained by analyzing the tissue concentration-depth profiles using eq. 1, as described in Methods. C_{avg} (average tissue concentration) was determined as the total amount of taxol found in tissue divided by the total tissue weight. C_u is urine concentration at 120 min and C_{pss} is the pseudo-steady-state plasma concentration

	C_u (μg/ml)	C_{uro} (μg/g)	C_{avg} (μg/g)	C_b (μg/g)	C_{pss} (ng/ml)	C_{uro}:C_u ratio	C_{avg}:C_u ratio	C_{uro}:C_{pss} ratio	C_{avg}:C_{pss} ratio	$w_{1/2}$ (μm)
Range	11.6–19.9	4.9–10.2	1.4–2.6	0.55–1.8	0.25–0.40	0.42–0.55	0.10–0.13	16 013–38 721	3388–9286	262–457
Median	14	7.8	1.5	0.86	0.32	0.49	0.12	19 366	5047	402
Mean ± SD	15.4 ± 4.2	7.5 ± 2.5	1.8 ± 0.6	1.0 ± 0.5	0.32 ± 0.05	0.48 ± 0.06	0.12 ± 0.01	24 562 ± 10 304	6056 ± 2524	381 ± 78

Fig. 2 Tissue concentration-depth profiles of taxol in the bladder after the 120-min treatment. Values are means + SD, $n = 13$. (● DMSO group, ○ water group). The data points are simply connected by straight lines. *Inset* A representative profile in one tissue section; the line is the computer-fitted line using the distributed model

previous findings with other drugs including mitomycin C, doxorubicin, and salicylate [3, 33, 36]. The remaining 10% was probably lost during experimental procedures, e.g. incomplete recovery of the urine sample. Note that the experimental procedures were designed to avoid damage to bladder tissues. Consequently it was not feasible to remove all residual urine.

Comparison of lipophilicity of taxol, mitomycin C and doxorubicin

The octanol:water partition coefficients were >99 for taxol, 0.52 for doxorubicin, and 0.41 for mitomycin C, indicating a rank order of lipophilicity of taxol > doxorubicin > mitomycin C.

Computer simulated tissue concentration-depth profiles of taxol, mitomycin C and doxorubicin

Figure 3 shows the simulated tissue concentration-depth profiles for the three drugs. The data show that the drug concentrations in tissue, as a function of the concentration in urine, were 14.7- to 26.7-fold higher for taxol compared with mitomycin C and doxorubicin at all tissue depths.

Table 3 Mass balance.The systemic bioavailability was estimated using the clearance and volume of distribution of taxol in humans, as described in the Methods

	Dose recovered in urine (%)	Dose recovered in bladder tissue (%)	Systemic bioavailability (%)	Total dose recovery (%)
Range	78–93	0.88–1.9	1.3–2.2	81–96
Median	85	1.2	1.8	89
Mean ± SD	86 ± 6.2	1.2 ± 0.4	1.7 ± 0.4	89 ± 6.1

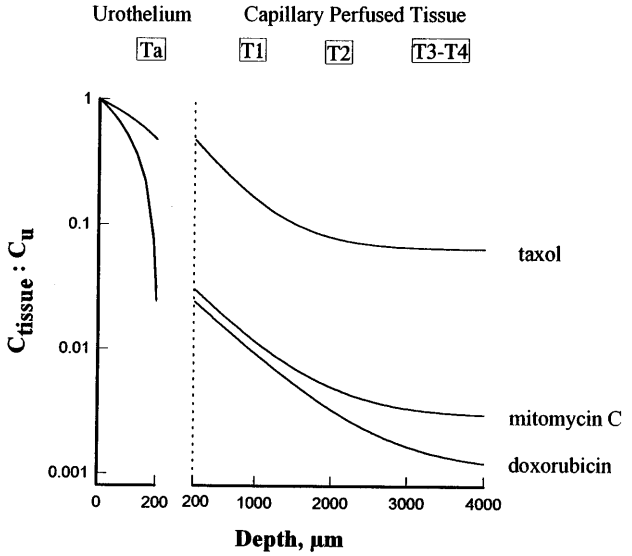

Fig. 3 Computer-simulated tissue concentration-depth profiles of taxol, mitomycin C, and doxorubicin. Tissue concentrations C_{tissue} were expressed as a function of urine concentration C_u at 120 min. Simulations were performed using bladder tissue pharmacokinetic parameters and eq. 1, as described in the Methods. Superficial tumors (*Ta* and *T1*) are located in the urothelium and the lamina propria, corresponding to 180 and 800 μm depths. Muscle-invading tumors (*T2*, *T3* and *T4*) are located in the superficial and deep muscle layers, corresponding to 2000 to 4000 μm depths

Discussion

The goal of the present study was to determine whether intravesical therapy with taxol provides a greater pharmacokinetic advantage than intravesical therapy with the currently used agents, namely mitomycin C and doxorubicin. Successful regional therapy requires high drug concentrations in tumor-bearing sites in order to achieve maximal antitumor effect, and low concentrations in the systemic circulation in order to minimize host toxicity.

The results of the present study showed that taxol readily penetrated the urothelium, as indicated by the high C_{uro}:C_u ratio of 0.5. In comparison, we have previously shown that other drugs currently used in intravesical therapy such as mitomycin C (MW 334 Da) and doxorubicin (MW 543 Da) have C_{uro}:C_u ratios of < 0.05 [33, 35, 36]. The higher transport of taxol across the urothelial barrier in spite of its higher molecular weight (MW 853 Da) suggests that molecular size is not a predominant determinant of drug transport across the urothelium. The more favorable transport of taxol is likely due to its higher lipophilicity, as indicated by its >200-fold higher octanol:water partition coefficient, compared with those for mitomycin C and doxorubicin.

The $w_{1/2}$ of taxol, i.e. ~400 μm, was slightly lower than but not significantly different from the $w_{1/2}$ of ~500 μm for mitomycin C and doxorubicin ($P > 0.1$). The value of $w_{1/2}$ is a function of drug removal by the perfusing capillaries (Eq. 1). The differences between the C_{uro}:C_u ratios and the lack of difference between the $w_{1/2}$ of these three compounds indicate that drug partition into the capillaries was less dependent on lipophilicity than partition across the urothelium. As a consequence of the higher C_{uro}:C_u ratio for taxol and the comparable $w_{1/2}$ values among these drugs, the C_{avg}:C_u ratio of taxol was significantly higher than those of mitomycin C and doxorubicin ($P < 0.05$). Accordingly, a much smaller dose was required to deliver therapeutic concentrations to bladder tissues in the case of taxol than in the cases of mitomycin C and doxorubicin. For example, at a 0.5 mg/8-kg dose, which is ~1% of the systemic intravenous infusion of 250 mg/m^2 over 24 h, the C_{avg} of taxol (~2 μg/g) was two to three times the peak plasma concentration achieved with intravenous administration (i.e. 0.75 μg/ml) [31]. For mitomycin C and doxorubicin, the C_{avg} achieved with intravesical administration at doses equivalent to intravenous doses is between 3 and 4 μg/g [35, 36], or three- to fourfold higher than the peak plasma concentrations delivered by an intravenous injection [9].

The estimated systemic bioavailability of $<2\%$ indicates that only a small fraction of the intravesical dose entered the systemic circulation. The plasma concentrations of taxol in dogs after intravesical instillation were $<0.05\%$ of the maximally tolerated plasma concentration of about 1 μg/ml in patients [14, 20, 21, 31], and the C_{avg}:C_u ratio was >6000:1 (Table 3). In comparison, doxorubicin shows a C_{avg}:C_{pss} of 1000 in humans [36]. Mitomycin C, which is not bound to tissues, shows a C_{avg}:C_{pss} of 246 in humans [35] and 46 in dogs [33]. Note that the tissue concentration represents the sum of free drug and tissue-bound drug, whereas the plasma concentration represents the sum of free drug and plasma protein-bound drug. Taxol appears to have different binding affinities to the proteins in plasma and in tissues; a previous tissue distribution study in animals showed a muscle:plasma concentration ratio of 23:1 after systemic injection [20]. In comparison, doxorubicin is bound to muscle with a muscle:plasma concentration ratio of about 22:1 [36], and mitomycin C is not bound

to tissues in appreciable amounts. When corrected for the differences in binding, the C_{avg}:C_{pss} is >300 for taxol, and ~50 for doxorubicin.

Collectively, the results of the present study indicate that intravesical taxol provides a significantly greater tissue targeting advantage than mitomycin C and doxorubicin which are commonly used in intravesical therapy. The present study was performed using water as the solvent for taxol. For clinical use, taxol is currently formulated in a vehicle of 50% ethanol and 50% polyethoxylated surfactant Cremophor EL. The choice of solvent can affect the drug delivery to the bladder. For example, a greater affinity of taxol for the solvent will result in a lower partition across the urothelium and into the bladder tissue. On the other hand, surface-active agents can alter the absorption barrier function of the urothelium. For example, dimethyl sulfoxide (DMSO) has been shown to enhance the bladder absorption of other drugs that are more hydrophilic than taxol, e.g. salicylate, cisplatin, pirarubicin and doxorubicin [5, 12, 25, 26]. DMSO presumably acts by widening the extracellular space [13, 15, 37].

This investigation is among a series of studies designed to evaluate the potential use of taxol by intravesical instillation to treat superficial and locally advanced bladder cancer. The results of the present study indicate that (a) intravesical instillation of taxol in water yielded significant drug concentration in bladder tissue, (b) the partitioning of taxol across the urothelium is more favorable than that of other commonly used agents, and (c) the systemic concentration of taxol resulting from intravesical treatment was insignificant in spite of the extensive absorption from the bladder. We conclude that a significant targeting advantage can be achieved using intravesical taxol therapy, and that taxol represents a viable candidate for intravesical therapy. Ongoing studies in our laboratory include the evaluation of taxol activity in histocultures of bladder tumor specimens from patients to determine whether the drug concentrations presented to deep muscle layers are adequate to produce a significant antitumor activity.

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