Bladder Tissue Pharmacokinetics and Antitumor Effect of Intravesical 5-Fluorouridine

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ABSTRACT

The present study evaluates whether intravesical 5-fluorouridine (FUR), a potent fluorinated pyrimidine, is effective against bladder cancer. The tissue and plasma pharmacokinetics of i.v. and intravesical FUR were studied in dogs to determine the tissue targeting advantage by the intravesical route. The i.v. study used a bolus FUR dose of 4 mg/kg, which is tolerated in humans. The disposition of FUR was biphasic, with a peak concentration of 8.8 μg/ml and a clearance of 127 ml/min/kg. 5-Fluorouracil was the major metabolite, reaching a peak concentration of 3.2 μg/ml. In the intravesical study, FUR (~2 mg/kg in 20 ml of water) was instilled in the dog bladder. At the end of the 2-h treatment, FUR concentration in urine decreased by about 40%, due mainly to dilution by residual and newly produced urine. The concentration at the interface between urothelium and lamina propria was 14 μg/g, or ~2% of the urine concentration, and declined logarithmically to 2 μg/g in the deep muscles. The concentrations of FUR and 5-fluorouracil in plasma were below the assay detection limit of 20 ng/ml, or >200-fold lower than the concentration after the i.v. dose (adjusted to the difference in the i.v. and intravesical dose). These data indicate a >200-fold advantage in the reduction of systemic exposure by the intravesical route. To determine whether the achievable tissue concentrations of FUR produced significant antitumor activity, we studied the effect of FUR against human bladder tumors maintained as 3-dimensional histocultures. The FUR concentrations (IC50s) required to produce 50% inhibition of DNA precursor ([3H]thymidine or bromodeoxyuridine) incorporation in human superficial bladder tumors (i.e., Ta and T1 tumors, n = 4) and muscle-invading tumors (i.e., T3 and T4 tumors, n = 4) were 9 and 22 μg/ml, respectively. In conclusion, intravesical FUR therapy delivers effective drug concentration to superficial bladder tissues without resulting in appreciable systemic blood concentration. We propose that intravesical FUR represents a potentially effective treatment against superficial bladder cancer.

INTRODUCTION

Of the 50,000 new cases of bladder cancer occurring each year in the United States, 80% present as superficial disease and 20% as invasive disease (1, 2). The standard therapy for superficial bladder cancer is transurethral tumor resection. But about 60% of patients will have recurrent tumors (1), which can be accompanied by a progression in grade and stage (3). The tumor recurrence may be due to unresected tumor cells, conversion of premalignant cells to malignant cells, and/or tumor regrowth. Adjuvant intravesical chemotherapy reduces the recurrence rate (4–6). The rationale of using intravesical chemotherapy is to selectively deliver high drug concentrations to the tumor-bearing bladder while minimizing the systemic exposure and thereby the systemic toxicity.

Intravesical chemotherapy has traditionally used DNA-damaging agents such as mitomycin C, doxorubicin, and thiota. Antimetabolites such as methotrexate have been used in systemic therapy of metastatic disease but not in intravesical treatment of localized disease. Because complete response is achieved in only 33–52% of patients treated with DNA-damaging agents (1), it is desirable to identify more effective drugs. The goal of the present study was to explore the use of antimeabolites for the intravesical treatment of bladder cancer. A comparison of antitumor activity of fluorinated pyrimidines in several tumor cell lines using the colony formation assay shows that FUR has higher activity than FU and 5-fluoro-2'-deoxyuridine on a molar basis (7, 8). Although the systemic toxicities of FUR prohibit its use as a systemic chemotherapeutic agent (9, 10), FUR may be a candidate for intravesical chemotherapy. The second reason for studying FUR is that its action is RNA directed rather than DNA directed and is, therefore, different from the traditionally used DNA-damaging agents. FUR is converted to 5-fluorouridine triphosphate, which is then incorporated into RNA, resulting in fraudulent RNA with altered function (11, 12). A third reason is that FUR and its active metabolite FU are rapidly eliminated. Because the pharmacokinetic advantage of regional therapy is linearly related to the clearance of the active agents after absorption into the systemic

Received 10/9/96; revised 2/13/97; accepted 2/18/97.

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1 Supported in part by Research Grant R37CA49816 from the National Cancer Institute, NIH, Department of Health and Human Services. The Ohio State University Tumor Procurement Service was supported in part by Cancer Center Support Grant P30CA16058 from the National Cancer Institute, NIH, Department of Health and Human Services.

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3 The abbreviations used are: FUR, 5-fluorouridine: BU, 5-bromouridine: FU, 5-fluorouracil: BrdUrd, 5-bromo-2'-deoxyuridine: CLU, 5-chlorouracil: ACN, acetonitrile; HPLC, high-performance liquid chromatography; LI, labeling index.
circulation (13), a higher clearance results in a higher pharmaco-kinetic advantage. As shown below, the systemic clearance of FUR was 2-4-fold higher than the clearance of mitomycin C and doxorubicin.

The present study evaluated the bladder tissue and plasma pharmacokinetics of intravesical FUR in dogs and the activity of FUR against patient bladder tumor histocultures to determine whether effective drug concentration can be delivered to the bladder wall. Dogs were chosen for this study because we found previously that the tissue pharmacokinetics of mitomycin C and doxorubicin in dog bladders are comparable to those in human bladders (14-16).

MATERIALS AND METHODS

Chemicals and Reagents. Chemicals and reagents FUR, BU, BrdUrd, and CLU were purchased from Sigma Chemical Co. (St. Louis, MO), ACN (HPLC grade) from Fisher Scientific Co. (Fair Lawn, NJ), sterile pigskin collagen (Spongostan standard) from Health Industries Design (Rochester, NY), [methyl-3H]thymidine from ICN Biomedicals (Irvine, CA), NTB-2 nuclear track emulsion from Eastman Kodak Co. (Rochester, NY), cefotaxime sodium from Hoechst-Roussel (Somerville, NJ), gentamicin from Solo Pak Laboratories (Franklin Park, IL), MEM from Life Technologies, Inc. Laboratories (Grand Island, NY), and antibody against BrdUrd from BioGenex (San Ramon, CA). Agents used for animal anesthesia and euthanasia were of USP grade. HPLC analysis showed that FUR, BU, and CLU were >99% pure. All reagents were used as received.

HPLC Equipment. Tissue and urine samples were analyzed by HPLC. The HPLC consisted of a Waters 510 solvent delivery pump, a Waters WISP 712 automated sample injector, a Waters model 441 UV detector (Waters Associates, Milford, MA), and a HP 3396A integrator (Hewlett-Packard Co., Wilmington, DE). Plasma samples were analyzed using a second HPLC consisting of a Hitachi model L-6200A Intelligent pump (Hitachi Instruments, Inc., Naperville, IL), a Waters 717 autosampler (Waters), an on-line precolumn filter (0.45 µm; Upchurch Scientific, Oak Harbor, WA), and an HP 1050 variable wavelength UV detector. The HPLC system was controlled by the HPLC Chemstation data system (Hewlett Packard).

Animal Protocol. Male and female beagle dogs (Hazelton Research Products, Inc., Kalamazoo, MI) weighing 9.4 ± 1.7 kg (n = 12) were used. The procedures were as described previously (14-17). In brief, animals were fasted overnight and allowed free access to water. A jugular vein was catheterized for the administration of the i.v. dose and the collection of systemic blood samples, and a cephalic vein was catheterized for the administration of anesthetics. Uretal catheters were inserted for the collection of urine samples and the administration of the intravesical dose. For the i.v. study, FUR (4 mg/kg) was given by a bolus injection over 0.5 min, and blood samples were taken over 4 h. For the intravesical study, the dog was sedated with a dose of acepromazine (0.56 mg/kg), followed 20-30 min later by an instillation of FUR (20 mg/20 ml) in the bladder through the urethral catheter. At about 115 min after drug instillation, the dogs were anesthetized. At 120 min, the urine was collected through the urethral catheter, and the bladder was removed. The bladder tissue was cut into left, right, and dome sections, and frozen as described previously (14-17).

Tissue and Urine Sample Analysis. Frozen tissue samples were cut in parallel to the urothelium surface into 40-µm slices using a cryotome (Carl Zeiss, Inc., Thornwood, NY), as described previously (14-17). The first 1-2 slices were contaminated by urine containing high drug concentration and were discarded. Subsequent slices were collected for analysis. The first 10 samples contained 1 slice each, the next 10 samples contained 2 slices each, and the remaining samples contained 4 slices each. A sample was placed in a preweighed 15-ml polypropylene centrifuge tube, spiked with 50 µl of the internal standard (BU, 80 µg/ml), and homogenized in 3 ml of ACN. The homogenizer probe was washed with 3 ml of ACN to recover residual tissue homogenate. The two ACN fractions were combined and centrifuged at 2000 × g for 5 min. The supernatant was transferred and evaporated to dryness in an evaporator (SpeedVac; Forma Scientific, Inc., Marietta, OH). The residue was reconstituted in 100 µl of water, vortexed, and sonicated for 2-3 min. Fifteen µl of the aqueous reconstituent was analyzed by HPLC using a reversed phase column (NovaPak C18; 3.9×300 mm, 4 µm; Waters), a mobile phase of 2.8% ACN in 2.5 mM ammonium acetate buffer adjusted to pH 5 with acetic acid, and UV absorption at 254 nm.

The extraction recoveries of FUR and BU from bladder tissue spikes at 5 concentrations between 35 and 212 ng of FUR and 4 µg of BU were 80.3 ± 14.0% and 85.5 ± 16.8%, respectively. The average coefficient of variation of the extraction recovery determined with three samples at each concentration was 3.6%. Urine samples were diluted 100-fold with distilled water, spiked with BU, and analyzed without extraction. FUR and BU were baseline separated from endogenous interferences. The HPLC elution times at a flow rate of 0.8 ml/min was 7.29 min for FUR and 16.0 min for BU. The standard curve of FUR in tissue was linear over the stated concentration range, with a coefficient of determination (r²) of 0.998 (n = 5).

Plasma Sample Analysis. Analysis of FUR and BU in plasma required a higher sensitivity than the tissue analysis and, therefore, the use of a second HPLC method. Plasma samples (0.5 ml) were spiked with 50 µl of internal standard (CLU, 470 µg/ml) and extracted using procedures described previously (18) with minor modifications. In brief, a sample was loaded on a LC-SCX solid-phase extraction column modified with 2 ml of methanol, 1 ml of 0.1 m copper(II) sulfate solution, and 3 ml of phosphate buffer (0.05 M, pH 7). After washing the column with 2 ml of phosphate buffer followed by 2 ml of methanol, the analytes were eluted with 1 ml of 1.7 mM ammonium solution and collected in a 15-ml polypropylene centrifuge tube containing 100 µl of glacial acetic acid. The eluant was then extracted with 2 fractions of 10 ml of ethyl acetate. The organic layer was transferred, evaporated to dryness in an evaporator, and reconstituted in 150 µl of water. Twenty to 100 µl were analyzed by HPLC, using a Nova-Pak C18 column (3.9×300 mm, 4 µm; Waters Associates), a mobile phase of 0.05% ACN in 50 mM ammonium acetate buffer adjusted to pH 4.5 with acetic acid, and UV detection at 268 nm. FU, FUR, and CLU were baseline separated from endogenous interferences with retention times of 7.3, 14.2, and 21.6 min, respectively, at a flow rate of 0.6 ml/min. The extraction recoveries of FU (20–5,000 ng/ml),...
FUR (20–10,000 ng/ml), and internal standard (CLU) from plasma were 81.4 ± 19.8%, 75.5 ± 14.9%, and 78.0 ± 14.7%, respectively (n = 5). The average coefficient of variation was 6.73%, determined at three different concentrations. The lower detection limit of FUR and FU was 20 ng/ml at a signal/noise ratio of 3 using 0.5 ml of plasma sample.

**Plasma Pharmacokinetic Data Analysis.** The plasma FUR concentration-time profiles obtained after an i.v. dose were analyzed using standard methods, assuming 2- or 3-compartment body models (19). The plasma concentrations of FUR achieved during the intravesical treatment were below the HPLC assay detection limit of 20 ng/ml (see “Results”), which precluded analysis of the absorption kinetics.

**Calculation of Upper Limit of Systemic Availability of Intravesical FUR.** The systemic availability of the intravesical dose equals the sum of the amount in the body at the end of treatment and the amount eliminated during treatment. The maximal amount of FUR in the body at the end of experiment was calculated as the product of \( FUR_{\text{min}} \) (detection limit of plasma FUR concentration) and \( V_d \) (volume of distribution). The amount of FUR cleared from the body during the 2-h treatment was calculated as the product of \( FUR_{\text{min}} \) (detection limit of plasma FUR concentration) \( \times C_l \) (clearance) \( \times (\text{instillation time, i.e.}, 120 \text{ min}) \). We further used the estimated systemically available intravesical dose as the infusion rate to calculate the upper limit of the pseudo steady-state plasma FUR concentration, as \( \text{Am}t_{\text{max}} \) (maximal amount absorbed systematically) \( \cdots C_l \) (clearance).

**Urine Pharmacokinetic Data Analysis.** Urine concentration-time profiles of FUR were analyzed using Eq. A, which has been used successfully to describe the urine pharmacokinetics of other drugs during intravesical treatment (14-16, 20, 21).

\[
C_u = \frac{\text{Dose}}{V_u} e^{-k_u t} + C_v
\]

where \( C_u \) is the urine concentration at time \( t \), \( V_u \) is the volume of the urine at time \( t \), \( k_u \) is a hybridized first-order rate constant describing drug absorption and degradation, \( V_v \) is the volume of dosing solution, \( k_d \) is the zero-order urine production rate, and \( V_{\text{res}} \) is the residual urine volume present in the bladder at the time of instillation.

**Tissue Pharmacokinetic Data Analysis.** 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 showed previously that bladder tissue pharmacokinetics of a drug are described by the distributed model (14–17) as follows:

\[
C_x = (C_{\text{uroth}} - C_h) e^{-D a x} + C_h
\]

where \( w_{1/2} = 0.693 \sqrt{D / \rho a} \) (B)

where \( x \) is the tissue depth, \( C_x \) is the concentration at depth \( x \), \( C_{\text{uroth}} \) is the concentration underneath the urothelium, and \( C_h \) is the concentration in the deep muscle layer, which is in equilibrium with the capillary plasma drug concentration, half-width (\( \sigma \)) is the depth over which the concentration declines by one-half, \( p \) is the capillary permeability, \( a \) is the capillary surface area per unit tissue volume, and \( D \) is the drug diffusion coefficient in the capillary perfused tissue layer.

**Tumor Histocultures.** Surgical specimens of primary human bladder tumors, obtained from chemotherapy-naïve patients during transurethral resection or cystectomy, were provided by the Tumor Procurement Service at The Ohio State University Comprehensive Cancer Center. Tumor stage and grade were determined according to the WHO classification (22). Tumor specimens were placed in Hank’s medium within 10–30 min after surgery and maintained at 4°C until use. Histoculture of tumors was performed as described previously (23–25). The advantage of human tumor histocultures are the use of human tissues, maintenance of the three-dimensional tissue architecture, stromal and tumor cells, cell-cell interactions, and cell heterogeneity. Furthermore, drug penetration in histocultures is more in vivo-like than in monolayer cell culture. We have shown that about 70% of human bladder tumors can be maintained as histocultures (24, 25). In brief, tumor specimens were sectioned to ~1-mm³ pieces. Four to six tumor pieces were placed on a 1-cm² presoaked collagen gel and cultured in six-well plates in a humidified atmosphere of 95% air and 5% CO₂ at 37°C. The culture medium was MEM supplemented with 9% heat-inactivated fetal bovine serum, 0.1 mM nonessential amino acids, 100 μg/ml gentamicin, and 95 μg/ml cefotaxime, with a pH of 7.4. After culture for 3 or 4 days, the tumors were used for pharmacodynamic studies.

**Pharmacodynamic Studies.** The antiproliferation effect was measured by the inhibition of DNA precursor incorporation.
Table I Pharmacokinetics of intravenous FUR

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>1/2a min</th>
<th>1/2ß min</th>
<th>α min⁻¹</th>
<th>β min⁻¹</th>
<th>C × Tµg/ml × min</th>
<th>Clearance ml/min/kg</th>
<th>Vdss ml/kg</th>
<th>Vf ml/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.07-1.77</td>
<td>18.2-33.3</td>
<td>0.391-0.648</td>
<td>0.021-0.038</td>
<td>21.9-47.4</td>
<td>84.4-183</td>
<td>627-1333</td>
<td>253-372</td>
</tr>
<tr>
<td>Median</td>
<td>1.47</td>
<td>25.4</td>
<td>0.480</td>
<td>0.030</td>
<td>34.9</td>
<td>115</td>
<td>680</td>
<td>277</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.46</td>
<td>26.1</td>
<td>0.491</td>
<td>0.028</td>
<td>34.2</td>
<td>127</td>
<td>799</td>
<td>288</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SD</td>
<td>0.29</td>
<td>5.9</td>
<td>0.103</td>
<td>0.007</td>
<td>10.4</td>
<td>41.3</td>
<td>273</td>
<td>48</td>
<td></td>
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</tr>
</tbody>
</table>

* C × T, area under concentration-time profile; Vdss and Vf, volumes of distribution at steady state and the volume of the central compartment, respectively.

Six dogs received an i.v. bolus injection of FUR (4 mg/kg). Plasma FUR concentration-time profiles were analyzed using a two-compartment body model.

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in tumor cells. A preliminary study in five bladder tumors showed that the two DNA precursors, BrdUrd and [3H]thymidine, labeled the same cells in all five tumors, resulting in identical labeling indices. In the present study, initial experiments in two tumors used [3H]thymidine. Later experiments in six tumors used BrdUrd to reduce the use of radioisotopes. Tissue sections were examined microscopically, the BrdUrd- or [3H]thymidine-labeled tumor cells were scored, and the fraction of labeled cells was determined. A typical experiment used a total of 7–15 tumor pieces for each drug concentration. Approximately 200 cells per tumor piece or >1400 cells were counted per concentration.

The relationship of FUR-induced inhibition of proliferation and drug concentration was analyzed by computer fitting the following equation to the experimental data:

\[ E = E_o \times \left(1 - \frac{C}{K + C}\right) \]  

where \( E \) is the LI of drug-treated tissues, \( C \) is the drug concentration, \( E_o \) is the LI of untreated controls, \( K \) is the drug concentration at one-half \( E_o \), and \( n \) is a curve shape parameter. The drug concentration that produced 50% inhibition (IC50) was determined.

Computer Simulation of Drug Concentration-Tissue Depth Profile. A comparison of the IC50s of tumors with the bladder tissue pharmacokinetics indicates whether the tumors are likely to receive effective drug concentrations for successful therapy. The theoretical basis for the tissue pharmacokinetic models and the experimental procedures for computer simulations are detailed in a previous publication (27). Because the urine concentrations and, thus, the tissue concentrations are not constant with time, the initial drug concentration could not be used as measure of drug exposure. A measure of cumulative drug exposure, i.e., product of concentration and exposure time \( C \times T \) was used instead. This was calculated as the time integral of concentration over the instillation time of 120 min. The effective drug exposures corresponding to 50% inhibition are denoted as \( C \times T_{50p} \).

Computer Simulations and Data Analysis. The simulations were done using an IBM-compatible, Intel Pentium-60 microprocessor-equipped personal computer (Gateway, North Sioux City, SD). Simulations used numerical integration, over 5-min discrete time intervals, programmed in SAS basic language. Nonlinear least square regression (SAS) was used to analyze the pharmacokinetic and pharmacodynamic data. Differences between groups were determined with the two-tailed Student’s t test. Akaike’s information criterion (28) was used to
compare the goodness of fit of data by alternative models, i.e., two-compartment versus three-compartment model.

RESULTS

Pharmacokinetics of i.v. FUR. i.v. FUR pharmacokinetics were established to calculate the upper limit of FUR absorption into systemic circulation during intravesical instillation and to provide an estimate of the targeting advantage of intravesical FUR administration.

Fig. 1 shows the plasma concentrations of FUR after an i.v. bolus dose. FUR was rapidly eliminated with FU as the major circulating metabolite. The peak FU concentration was obtained in the first sample at 1 min. FU concentrations were higher than FUR concentrations and declined in parallel with the FUR concentrations. The FUR and FU concentration in the 2- and 4-h samples were below the detection limit of 20 ng/ml. Analysis by Akaike’s Information Criterion shows that the plasma FUR concentration-time profiles were better described by a two-compartment model than by a three-compartment model. Table 1 summarizes the pharmacokinetic parameters of FUR.

Urine and Plasma Pharmacokinetics of Intravesical FUR. Fig. 2 shows the mean urine concentration-time profiles of FUR after the intravesical dose. Table 2 summarizes the urine pharmacokinetic parameters. The concentration decline in the first 5 min (11%) was mainly due to the dilution of the 20-ml dosing solution by the residual urine (~3 ml). The decline in the subsequent 115 min was slower (29%) and was due to further dilution by the urine produced during treatment and drug removal by absorption and/or degradation. No HPLC peaks corresponding to FU were observed, indicating that FUR was not degraded to FU in the urine.

Systemic plasma concentrations of FUR and FU in all animals were below the detection limit of the HPLC assay (20 ng/ml) at all time points. A mass balance evaluation indicates that a total of ~90% of the dose was recovered in the 120-min urine sample and bladder tissue (Tables 1 and 2). Because FUR and FU are eliminated mainly by metabolism and not by urinary excretion, the dose fraction in urine was not likely to derive from the drug/metabolite absorbed systemically. Accordingly, the upper limit of the intravesical dose absorbed into the systemic circulation is 10%, which would yield a calculated steady-state plasma concentration (C_{ss}) of ~10 ng/ml.

Tissue Pharmacokinetics of Intravesical FUR. Each bladder was divided into three sections, i.e., dome, and right and left lateral sides. There was no apparent relationship between the anatomical location and drug penetration characteristics (data not shown), similar to our previous findings with mitomycin C and doxorubicin (14–16). Fig. 3 shows the FUR concentration in bladder tissue as a function of depth from the urothelium. As it penetrated the capillary periphery layer at the end of the 120-mm treatment. Table 3 shows the tissue pharmacokinetic parameters. The drug concentration at the interface of the urothelium and the lamina propria was about 2% of the urine concentration, indicating a limited absorption of FUR across the urothelium. As it penetrated the capillary perfused tissues, including the lamina propria and the superficial and deep muscularis, the FUR concentrations declined logarithmically with a w of ~400 μm and reached a minimum plateau level of about 2 μg/g at 2000-μm depth.

The total amount of FUR in each bladder, calculated by summing the drug amounts in all tissue segments, was 22.8 ± 12.9 μg (mean ± SD of six dogs). This equals 0.1% of the dose.

Pharmacodynamics of FUR in Human Bladder Tumors. Eight tumors, including four superficial (T1 and T2) and four muscle-invading (T3 and T4) tumors, were successfully cultured and used to evaluate the antitumor activity of FUR.
Table 3  Bladder tissue pharmacokinetics of intravesical FUR

Six animals received an intravesical dose of FUR (20 mg in 20 ml of water) for 120 min. After treatment, the bladder was removed and divided into three sections (dome and left and right lateral regions), and two to three sections per animal were analyzed for drug concentrations. Data represent a total of 16 tissues. The tissue concentration-depth profiles were analyzed using Eq. B to obtain the tissue pharmacokinetic parameters. $C_{av}$, concentration in the urothelium; $C_{eq}$, average tissue concentration; $C_{eq}$, equilibrating deep tissue concentration; $w_{1/2}$, half-width.

<table>
<thead>
<tr>
<th>Tumor</th>
<th>Patient gender/age</th>
<th>Surgical procedure</th>
<th>Stage</th>
<th>Grade</th>
<th>Control LI (%)</th>
<th>$IC_{50}$ (µg/ml)</th>
<th>$E_{max}$ at 100 µg/ml (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial</td>
<td>1</td>
<td>F/76</td>
<td>TUR*</td>
<td>II</td>
<td>50.2</td>
<td>4.1</td>
<td>85</td>
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<tr>
<td></td>
<td>2</td>
<td>M/64</td>
<td>TUR</td>
<td>II</td>
<td>46.9</td>
<td>23.2</td>
<td>75</td>
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<td></td>
<td>3</td>
<td>M/69</td>
<td>Cystectomy</td>
<td>T1</td>
<td>II-III</td>
<td>80.8</td>
<td>8.2</td>
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<tr>
<td></td>
<td>4</td>
<td>M/54</td>
<td>Cystectomy</td>
<td>T2</td>
<td>III</td>
<td>34.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>8 M/85</td>
<td>Cystectomy</td>
<td>T2</td>
<td>III</td>
<td>44.0 ± 16.3</td>
<td>21.7 ± 10.7</td>
<td>67 ± 4.7</td>
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<tr>
<td>Muscle invading</td>
<td>5</td>
<td>M/65</td>
<td>Cystectomy</td>
<td>T3</td>
<td>IV</td>
<td>55.5</td>
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<td></td>
<td>6</td>
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<td>37.2</td>
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<tr>
<td></td>
<td>7</td>
<td>F/65</td>
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<td>III</td>
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<td>13.9</td>
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<tr>
<td>Mean ± SD</td>
<td>8 F/85</td>
<td>Cystectomy</td>
<td>T3</td>
<td>III</td>
<td>44.0 ± 16.3</td>
<td>21.7 ± 10.7</td>
<td>67 ± 4.7</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0.51</td>
<td>0.13</td>
<td>0.01</td>
</tr>
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</table>

* TUR, transurethral resection; NA, not applicable.

Table 4  Patient and tumor characteristics and tumor sensitivity to FUR

There was no difference between the labeling indices of the untreated superficial and muscle-invading tumors (Table 4). Fig. 4 shows the relationship between drug concentration and inhibition of DNA precursor incorporation. The inhibition increased with drug concentration in a classical sigmoidal relationship. However, at the highest concentration of 100 µg/ml, the inhibition was complete only for one tumor and was between 62 and 91% for the remaining seven tumors. The superficial tumors showed a significantly higher maximum inhibition than the invasive tumors (Table 4). The $IC_{50}$ appeared to be lower for superficial tumors than invasive tumors, but the difference was not statistically significant. No obvious FUR-induced morphological changes (i.e., necrosis or apoptosis) were observed.

**Comparison of Effective $C \times T_{50}$ Values with Drug Exposure Achieved in Bladder Tissues.** Tumors of different stages are located in different tissue depths in the bladder wall, i.e., $T_n$ tumors are in the urothelium, $T_1$ tumors in lamina propria, $T_2$ tumors in superficial muscle layers, and $T_3$ and $T_4$ tumors in deep muscle layers. Fig. 5 compares the drug exposure achieved at different tissue depths in human bladders with the effective $C \times T_{50}$ for tumors of different stages. The drug exposure in the urothelium exceeded the $C \times T_{50}$ of the one $T_3$ tumor, indicating that this tumor would have received sufficient drug exposure to produce a 50% inhibition. On the other hand, only one of the three $T_1$ tumors and none of the $T_3$ and $T_4$ tumors received $C \times T_{50}$.

Fig. 4  Pharmacodynamics of FUR in bladder tumor histocultures. Tumor histocultures were treated with FUR for 2 h. After drug treatment, tumor samples were exposed to $[^3H]$thymidine and processed for autoradiography, or to bromodeoxyuridine and processed for immunohistochemical detection. Data represent means; bars, 1 SE (or = 4). Lines represent the model-fitted lines using Eq. C: *, superficial tumors; O, muscle-invading tumors.
The parallel decline of the concentrations of FUR and FU indicates that FU was eliminated at an equal or more rapid rate (e.g., propranolol and its metabolite naphthoxylacetic acid) after iv. injection (39). The concentration-time profile of FU did not follow the peak concentration followed by a decrease. Instead, the peak FU concentration was reached in the first sample at 1 min, indicating near-instantaneous conversion of FUR to FU. The parallel decline of the concentrations of FUR and FU indicates that FU was eliminated at an equal or more rapid rate than the parent drug. This is consistent with the shorter elimination half-life of FU (9.4 min) in dogs (40) as compared to FUR (26 min; Table 1). On a molar basis, the FU concentrations in plasma derived from the intravesical dose is one-half of 34 μg-mm/ml. From Table 1, the IC50 of FUR in bladder tumors was between 1 and 37 μg/ml, or 3.8 to 141 μM. The mean IC50 values (58 μM) are 4- and 8-fold higher than the IC50 of mitomycin C and doxorubicin, i.e., 12.6 (range, 0.7–44.6) and 6.5 (range, 0.14–

Fig. 5  Comparison of cumulative drug exposure achieved at different tissue depths and effective C × T0.5 values. Mean cumulative drug exposure was calculated as the integral of mean drug concentration over time (C × T). The profile was computer simulated using the urine pharmacokinetics and tissue pharmacokinetic parameters, as described in "Materials and Methods." The C × T0.5 values for tumors of different stages were plotted according to their respective anatomical sites.

DISCUSSION
Pharmacokinetics of i.v. FUR. Although there are several antitumor and toxicological studies of FUR in rodents (10, 29–32) and humans (33), no pharmacokinetic data are available in the literature. The present report represents the first i.v. pharmacokinetic study of FUR. The clearance of FUR in dogs equals cardiac output (120 ml/min/kg; Ref. 34). This high clearance is consistent with the known metabolic pathway and the rapid metabolic rate (see below and Refs. 35 and 36); FUR is metabolized to FU by uridine phosphorylase, a ubiquitous enzyme present in tissues and plasma (37, 38).

The concentration-time profile of FU did not follow the traditional metabolite kinetic profile that shows an increase to peak concentration followed by a decrease. Instead, the peak FU concentration was reached in the first sample at 1 min, indicating near-instantaneous conversion of FUR to FU. Similar profiles have been observed for other drugs (e.g., propranolol and its metabolite naphthoxylacetic acid) after i.v. injection (39). The parallel decline of the concentrations of FUR and FU indicates that FU was eliminated at an equal or more rapid rate than the parent drug. This is consistent with the shorter elimination half-life of FU (9.4 min) in dogs (40) as compared to FUR (26 min; Table 1). On a molar basis, the FU concentrations were 1.4-fold higher than the FUR concentration. This was due to the 2–3 times lower clearance and smaller volume of distribution for FU in dogs when compared to FUR (clearance is 54 ml/min/kg, distribution volume at steady state is 290 ml/kg, and the volume of central compartment is 161 ml/kg; Ref. 40).

Pharmacokinetic Advantage of Intravesical FUR. The intravesical dose of FUR used in the present study is one-half of the i.v. dose of 4 mg/kg that has been used in patients (33). However, the intravesical treatment resulted in undetectable plasma concentration (i.e., <20 ng/ml). On the basis of the calculated upper limit of 10 ng/ml for Cmax, we estimated that the Cavg in bladder tissue is ≥500 times the Cmax, and that the maximum C × T in plasma derived from the intravesical dose is 1.2 μg-min/ml or <7% of the dose-adjusted C × T after the i.v. dose (i.e., one-half of 34 μg-min/ml). These data indicate that intravesical administration resulted in a substantial advantage compared to i.v. administration by delivering high drug concentration to bladder tissue with insignificant systemic drug concentration.

In the present study of dogs, we were able to minimize the variability in several parameters (e.g., urine production rate, residual urine volume, and intravesical pressure) that can potentially alter the urine concentration-time profile by restricting fluid intake during the 2-h study. We have shown that these parameters significantly alter the urine and tissue pharmacokinetics of mitomycin C and doxorubicin in patients (14–16), and that these parameters need to be carefully monitored during clinical evaluation.

Comparison of FUR with Mitomycin C and Doxorubicin. The bladder tissue penetration characteristics of FUR were remarkably similar to those of mitomycin C and doxorubicin, the two drugs commonly used in intravesical treatment of superficial bladder cancer. All three drugs showed a low Cmin/Cmax ratio, i.e., 0.02 for FUR, 0.03 for mitomycin C, and 0.02 for doxorubicin; and showed a logarithmic decline in concentration in the capillary-perfused bladder tissues, with w- values within a relatively narrow range of 419 for FUR, 484 for mitomycin C (15), and 534 for doxorubicin (16). w- is a function of the drug removal by the perfusing blood. The similar Cmin/Cmax ratios and w- values of the three drugs indicate that the partition across the urothelium and penetration into capillaries were not affected significantly by the different molecular weights of these drugs (262 daltons for FUR, 334 daltons for mitomycin C, and 544 daltons for doxorubicin).

The pharmacokinetic advantage of a regional intra-organ therapy is linearly proportional to the systemic drug clearance and inversely proportional to blood flow (13). If drug elimination takes place in the organ, the pharmacokinetic advantage is also inversely proportional to (1 – extraction ratio of drug in organ). In dogs, FUR, mitomycin C, and doxorubicin have similar w- and similarly negligible extraction by the bladder, as indicated by the recovery of ≥85% of the dose in urine and bladder tissue (15). On the other hand, the clearance of FUR in dogs (127 ml/min/kg) is 4.4- and 2.2-fold higher than the clearances of 24 ml/min/kg and 40 ml/min/kg for mitomycin C (41) and doxorubicin (42), respectively. Accordingly, the pharmacokinetic advantage of intravesical FUR is 2–4-fold higher than the other two drugs.

Antitumor Activity of FUR. Data in Table 4 indicate a trend of a higher sensitivity of superficial tumors to FUR compared to muscle-invading tumors, but the difference was not statistically significant, probably because of the relatively small sample size. The IC50 of FUR in bladder tumors was between ~1 and 37 μg/ml, or 3.8 to 141 μM. The mean IC50 values (58 μM) are 4- and 8-fold higher than the IC50 of mitomycin C and doxorubicin, i.e., 12.6 (range, 0.7–44.6) and 6.5 (range, 0.14–
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>100) μM, respectively (25, 26). This indicates that FUR has lower potency than the other two drugs after a 2-h treatment. Interestingly, tumors treated with FUR did not show altered morphology. In contrast, necrotic and apoptotic morphologies were observed for mitomycin C and doxorubicin (25, 26). The causes of the lower chemosensitivity of human bladder tumors to FUR relative to mitomycin C and doxorubicin and the different morphologies of cells treated by the three drugs are not apparent. Possible reasons include: (a) differences of retention of these compounds in cells, e.g., doxorubicin via its binding to macromolecules is better retained in cells (43, 44); (b) different action mechanisms; (c) different time dependency in drug-induced antitumor effect; and (d) inefficient metabolism of FUR to its toxic triphosphate metabolite. Additional studies are needed to determine whether there is a relationship between tumor pathology and chemosensitivity, as was found for mitomycin C (45), and to determine whether bladder tumors are inherently less sensitive to antimetabolites than to DNA-damaging agents.

**Usefulness of FUR in Intravesical Treatment of Bladder Cancer.** The efficacy of intravesical chemotherapy is determined by the drug concentration at the tumor site and the chemosensitivity of tumor cells, whereas the treatment-related local and systemic toxicity is determined by the sensitivity of normal bladder tissue and by the systemic drug concentration. The latter is in turn determined by the absorbed dose fraction and the drug clearance. As discussed above, FUR does not offer an advantage over mitomycin C and doxorubicin in bladder tissue penetration or in chemosensitivity. On the other hand, the higher pharmacokinetic advantage of FUR because of its higher clearance makes it an attractive alternative agent, especially when significant absorption of the intravesical dose is anticipated, e.g., when the barrier function of the urothelium is compromised due to pathological conditions, surgical manipulation, or the use of absorption enhancers. We have shown a significantly higher absorption of mitomycin C in patients when the drug was instilled within days after transurethral bladder tumor resection than when it was instilled after more than 1 week (21). A second advantage of FUR is its apparent lack of direct toxicity on cells, as indicated by the absence of morphological changes. This may reduce the potential local toxicity that is seen with other agents, e.g., doxorubicin (1, 4). A third advantage of FUR is that because of its unique RNA-directed mechanism, FUR represents an attractive candidate for use in combination with other drugs that have different mechanisms of action.

The present study addressed the chemosensitivity of bladder tumor after a single treatment. Intravesical chemotherapy is often given weekly for 6 weeks. It is conceivable that drug efficacy is substantially higher after repeated treatments. Furthermore, the present study selected a dose that is equivalent to one-half of the i.v. dose that was used in humans, at a concentration (i.e., 20 μg/20 ml water) that is equivalent to the concentration of other currently used agents. Judging from the minimal systemic concentration of FUR and its active metabolite FU, it may be feasible to increase the concentration of the dosing solution. Further studies are also needed to determine the optimal treatment schedules, preferably in preclinical *in vivo* bladder tumor models.

**Conclusions.** In summary, data of the present study indicate that intravesical FUR treatment is likely to have a greater pharmacokinetic advantage over the commonly used DNA-directed agents, such as mitomycin C and doxorubicin, and is effective against superficial bladder tumors. FUR represents an attractive choice for further development. Ongoing studies in our laboratory evaluate the use of absorption enhancers to increase the FUR penetration in muscle layers and the use of FUR in combination with other agents.

**ACKNOWLEDGMENTS**

We thank Xiang Gao for assistance in animal experiments and Li-Fen Hsu for assistance in tissue sample preparation.

**REFERENCES**


Bladder tissue pharmacokinetics and antitumor effect of intravesical 5-fluorouridine.

D Song, M G Wientjes, Y Gan, et al.


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