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ORIGINAL ARTICLE

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Cremophor reduces paclitaxel penetration into bladder wall during intravesical treatment

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Abstract *Purpose*: We have previously shown that paclitaxel, when dissolved in water and instilled into the bladder, readily penetrates the urothelium. The FDA-approved formulation uses Cremophor and ethanol to dissolve paclitaxel. In the present study, the effects of this solvent system on the urine, bladder tissue, and plasma pharmacokinetics of intravesical paclitaxel were evaluated. *Methods*: Plasma, urine, and tissue pharmacokinetics were determined in five dogs treated for 120 min with paclitaxel (500 μg per 20 ml of 0.22% w/v Cremophor and 0.21% v/v ethanol) by intravesical instillation. Equilibrium dialysis was used to determine the free fraction of paclitaxel and the presence of Cremophor micelles was verified using a fluorescent probe method. *Results*: The average bladder tissue concentration was >1600-fold higher than the plasma concentration. Comparison of the results for paclitaxel dissolved in Cremophor/ethanol with our previous results of paclitaxel dissolved in water (500 μg per 20 ml) indicates that Cremophor/ethanol decreased the paclitaxel partition across the urothelium and reduced the average bladder tissue concentration by 75%, but did not alter the rate of paclitaxel penetration across the bladder wall, the urine pharmacokinetics or the plasma pharmacokinetics of paclitaxel. For Cremophor, the urine concentrations during the 120-min treatment ranged from 0.12% to 0.22%, and the concentration in bladder tissue from 0.00004% to 0.0009%. The threshold Cremophor concentration for micelle formation was 0.008%. We found that ethanol at concentrations up to 1% and Cremophor at concentrations below 0.01% did not alter the free fraction of paclitaxel, whereas Cremophor at higher concentrations, i.e. 0.065% and 0.25%, significantly reduced the free fraction by two- to six-fold, respectively. These results indicate that during intravesical instillation of the FDA-approved paclitaxel formulation, the concentration of Cremophor in urine was sufficient to form micelles, resulting in sequestration of paclitaxel into micelles, reduction in the free fraction of paclitaxel and consequently a reduction in paclitaxel penetration across the urothelium. In contrast, the Cremophor concentrations in bladder tissue were inadequate to form micelles and thus did not alter the drug penetration through the bladder tissue. *Conclusions*: We conclude that intravesical paclitaxel treatment using the FDA-approved formulation provides a significant bladder tissue targeting advantage, although the advantage is lower than when paclitaxel is dissolved in water.

Key words Paclitaxel · Bladder cancer · Cremophor · Intravesical therapy

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Introduction

Management of superficial bladder cancer usually consists of transurethral tumor resection of visible tumors and margins followed by adjuvant intravesical chemotherapy to reduce recurrence and/or progression [11]. Intravesical chemotherapy presents a targeting advantage by selectively delivering the drug in high concentration to the tumor-bearing bladder tissue while minimizing systemic exposure.

The pharmacologic and biologic basis for the variable response of bladder cancer after intravesical chemotherapy has been investigated in our laboratory [2, 4, 29, 31]. Our data suggest that treatment failure is in part due to the inability of the currently used drugs to penetrate the deeper tissue and in part due to the low sensitivity of the more rapidly proliferating tumors to these drugs. Logical approaches to developing more effective intravesical chemotherapy are to identify drugs active against the aggressive tumor and drugs that readily penetrate through the urothelium into the deep tissue.

Paclitaxel is one of the most important anticancer agents developed in the last two decades, and has shown significant activity against multiple types of human solid tumors, both in histoculture and in patients, including carcinoma of the head and neck, ovary, bladder, lung, and esophagus [8, 17, 21]. In patient bladder tumors, paclitaxel inhibits tumor cell proliferation and induces apoptosis, with a greater apoptotic effect in the more rapidly proliferating tumors [1]. Furthermore, our previous study in dogs has shown that intravesical administration of paclitaxel, when dissolved in water, produces a substantial targeting advantage with a 6000-fold higher average bladder tissue concentration compared to the steady-state plasma concentration [24]. These results indicate that paclitaxel is a viable candidate for intravesical therapy of bladder cancer, especially for the rapidly proliferating tumors which are relatively insensitive to the currently used drugs such as mitomycin C and doxorubicin [9, 22].

Paclitaxel is poorly soluble in water. The FDA-approved formulation uses micellar solubilization to dissolve paclitaxel to attain the relatively high concentration of 6 mg/ml; the solvent system consists of Cremophor (i.e. polyoxyethyleneglycerol triricinoleate 35 which is a derivative of castor oil) and ethanol. Nonionic surfactants such as Cremophor, at levels above the critical micellar concentration (CMC), can form micelles. Micelles are stable aggregates of surfactant monomer [27]. The partitioning and/or adhesion of lipophilic drugs into or onto micelles, may reduce the drug partitioning across biological membranes by reducing the fraction of diffusible drug (reviewed in reference 7).

In the present study, the urine, plasma and bladder tissue pharmacokinetics of paclitaxel and Cremophor, after administration of the FDA-approved formulation, were evaluated. The results were compared with our previous results obtained using paclitaxel dissolved in water [24], to determine the effect of Cremophor/ethanol on paclitaxel partitioning across the bladder urothelium and penetration across the bladder wall. The dog was chosen as the animal model because penetration of other drugs in dog bladders is comparable to that in patients [2, 29, 31].

Materials and methods

Chemicals and equipment

Paclitaxel was a gift from Bristol Myers Squibb (Wallingford, Ct.). Cephalomannine, 3″-[^{3}H]paclitaxel (specific activity, 19.3 Ci/mmol), and 7-epitaxol were from the National Cancer Institute (Bethesda, Md.). Ricinoleic acid, α-naphthylamine, 8-analino-1-naphthalenesulfonic acid (ANSA), oxalylchloride, and Cremophor were purchased from Sigma Chemical Company (St. Louis, Mo.). USP grade agents used for anesthesia and euthanasia, and high-performance liquid chromatographic (HPLC) grade chemicals were purchased from Fisher Scientific Company (Fair Lawn, N.J.) or Mallinkrodt Baker (Paris, N.Y.). The competitive inhibition enzyme immunoassay kit for taxanes was obtained from Hawaii Biotechnology Group (Aiea, Hawaii). HPLC analysis showed that paclitaxel, cephalomannine, and 7-epitaxol were >99% pure. All reagents were used as received.

Animal protocol

Male or female beagle dogs (Hazelton Research Products, Kalamazoo, Mich.) weighing 8.75 ± 0.58 kg (mean ± SD, $n = 5$) were used. Animals were fasted overnight and allowed access to water *ad libitum*. The surgical procedures were as described elsewhere [30]. An angiocatheter (20 G, 4 inch) was inserted in the cephalic vein for administration of anesthetics, and a second angiocatheter (16 G, 5.25 inch) was placed in the right jugular vein for blood sampling. French Foley catheters (size 13 or 14 for female dogs, and size 8 or 10 for male dogs) were inserted for dose instillation and sampling of the bladder contents. Animals received a sedative, acepromazine (0.5 mg/kg), by intravenous injection to facilitate catheterization. All experiments were started between 1 and 3 pm. Animals were anesthetized for the duration of the experiment. After emptying urine from the bladder, an intravesical dose of paclitaxel was instilled via the Foley catheter. The intravesical dose was prepared by diluting the commercially available clinical formulation (containing 6 mg paclitaxel, 527 mg Cremophor, and 0.447 ml ethanol per ml dosing solution) with water to yield a 20-ml solution with a final concentration of 500 μg paclitaxel in 0.22% w/v Cremophor and 0.21% v/v ethanol. The catheter was then flushed with air to ascertain complete delivery of the intravesical dose. Serial blood and urine samples were collected before and during the instillation period and immediately before surgical removal of the bladder. To obtain urine samples, a 10-ml syringe was connected to the outlet of the Foley catheter, and 5-ml of the bladder contents were withdrawn into the catheter. The latex catheter was then pierced with a 1-ml syringe (30 G needle), and samples (100 μl each) were taken. The contents of the catheter were then completely returned to the bladder. By this method, the collection of a urine sample was completed within 2 min.

At the end of the 120-min treatment period, the bladder was exposed by a midline incision, and the dome and left and right lateral sides of the bladder were marked with superficial stitches of surgical silk. In order to maintain the concentration gradient between urine and tissue and to avoid washout of the drug during processing of the tissue, it was necessary to maintain the drug solution in the bladder until just prior to clamping the blood supply. After draining the bladder contents at 120 min, the urethral arterial blood supply was clamped, and the bladder was rapidly removed. Tissue sections of approximately 2 × 2 cm surface area were cut from the dome and left and right lateral sides. Tissue sections were placed on a flat stainless-steel plate (urothelial side on the plate) cooled on dry ice, and snap-frozen in liquid nitrogen. A short time interval between clamping the blood supply to the bladder and freezing the bladder tissue is critical for obtaining accurate tissue concentration-depth profiles [31]. The procedures between removing the intravesical dose and freezing the bladder tissue specimens required less than 5 min. Animals were euthanized with an overdose of intravenous pentobarbital immediately after removal of the bladder.

Analysis of paclitaxel

The concentrations of paclitaxel in bladder tissue and urine samples were analyzed by a previously described HPLC assay which is specific for paclitaxel and its active metabolite 7-epitaxol [20, 23] and has a lower limit of detection of 5 ng/ml for urine samples and 5 ng/injection for bladder tissue samples. The concentration of paclitaxel in plasma samples, because of the low level, was analyzed by immunoassay. The immunoassay measures all taxanes and does not distinguish paclitaxel from its metabolites [10], and has a lower detection limit, after procedural adjustment described in methods, of 0.12 ng/ml.

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 [30]. The first and/or second sections were discarded to rule out contamination of tissues by urine. The next ten sections were analyzed separately, whereas the subsequent sections, because they contained lower drug concentrations, were pooled for analysis. In addition, samples were consistently alternated for analysis either for paclitaxel or stored frozen at −70 °C for subsequent determination of Cremophor concentration.

Analysis of paclitaxel by immunoassay was as described previously [24]. To improve assay sensitivity, paclitaxel was extracted from plasma using 3 × 3 ml ethyl acetate. Standard curves encompassing a drug concentration range of 0.12 to 90 ng/ml were prepared daily and analyzed simultaneously with samples. The standard curves were sigmoidal in shape; concentrations between 0.36 and 10 ng/ml were within the linear range. The intraday variation ranged from 2% to 7%.

HPLC analysis of Cremophor

Analysis of Cremophor, which does not absorb UV light, required conversion to ricinoleic acid (i.e. *N*-ricinoleoyl-1-naphthylamine) [26]. Briefly, saponification of Cremophor by hydrolysis under alkaline conditions produced substituent fatty acids of which 87% was ricinoleic acid. The free fatty acids were extracted with chloroform followed by derivatization with 1-naphthylamine. Analysis of tissue samples required additional sample processing, as follows. Preweighed tissue samples were vortex-mixed with 30 μl internal standard (margaric acid, 2.01 mg/ml) and 1 ml chloroform until all tissue floated freely in solution. The solution was quantitatively transferred to a 15 ml nalgene culture tube. An additional 4 ml of chloroform was added. The mixtures were incubated at 37 °C with continuous shaking for 120 min, supplemented with vortex-mixing every 15 min. After centrifugation at 2000 *g* for 15 min at 4 °C, the aqueous supernatant was removed by suction. The remaining organic layer was quantitatively transferred to a 10-ml glass tube with PTFE-lined screw-cap and dried under nitrogen. The residue was reconstituted in 200 μl methanol/acetonitrile/water (72:13:15, v/v/v), and 100 μl of the reconstituted derivatized product was analyzed by HPLC. Because the concentrations of Cremophor in individual tissues were below the detection limit, multiple samples were combined for analysis. Standard curves were constructed using untreated dog bladder tissues and were fitted using quadratic regression analysis. No interferences were observed in the standard curve samples.

Analysis of tissue concentration-depth profiles of paclitaxel and Cremophor

We have shown that drug penetration in bladder follows the distributed model which describes drug removal by capillary drainage in addition to drug diffusion [2, 24, 25, 29–31]. The decline in drug concentrations with respect to the tissue depth is described by Eq. 1:

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

where C_0 is the drug concentration at the beginning of the capillary-perfused tissue which is the interface between urothelium and lamina propria (about 50 μm deep by microscopic examination), C_b is the drug concentration in the deepest tissue layer, C_x is the concentration at distance x into capillary tissue, and $w_{1/2}$ is the tissue thickness over which the concentration declines by 50%.

Analysis of concentration-time profiles of paclitaxel in urine

The concentration-time profiles of paclitaxel in urine during the 120 min instillation period were analyzed using Eqs. 2 and 3:

$$C_u = \frac{\text{Dose}}{V_u} e^{-(k_a + k_d)t} \quad (2)$$

$$V_u = V_0 + k_0 t + V_{res} \quad (3)$$

where V_u is the volume of urine at time t, V_0 is the volume of paclitaxel dosing solution (20 ml), k_0 is the zero-order rate constant for urine production, V_{res} is the volume of residual urine present in bladder at time of dose instillation, k_a is the first-order rate constant for drug absorption into the systemic circulation, and k_d is the hybridized first-order rate constant describing degradation, metabolism, and tissue binding of paclitaxel. In this study, systemic blood concentrations of paclitaxel were at least five orders of magnitude lower than the urine concentrations. Hence, the transfer of paclitaxel from the systemic circulation to the bladder (i.e. urinary excretion) was considered negligible and was not included in the model. The average drug concentration in bladder tissue was determined as the total amount of paclitaxel found in tissue divided by the total tissue weight. Tissue concentrations were plotted against the midpoint depth of the tissue section. The fraction of the dose recovered was calculated as the amount recovered in urine collected through the Foley catheter divided by the amount administered. The value k_0 in Eq. 3 was calculated as the difference between the volume of the dosing solution (20 ml) and the volume of the urine recovered at 120 min (V_{120}) (Eq. 4):

$$k_0 = \frac{V_{120} - 20}{120} \quad (4)$$

Effect of Cremophor and ethanol on the free fraction of paclitaxel

The free fraction of paclitaxel was determined by equilibrium dialysis at 37 °C, using a Spectrum side-by-side equilibrium dialysis apparatus. The donor compartment contained various concentrations of Cremophor and/or ethanol, and a constant ratio of unlabeled and tritium-labeled paclitaxel. The receiver compartment contained only phosphate-buffered saline (PBS). The final specific activity of 3′′-[^{3}H]paclitaxel was 0.19 Ci/mmol. The two compartments were separated by a Spectra/Por cellulose acetate membrane. As shown in the Results, paclitaxel was entrapped in Cremophor micelles. Hence it was necessary to use a dialysis membrane with a molecular weight cut-off of 1000 Da, to separate paclitaxel (MW 853 Da) and Cremophor (MW 1680 Da). A preliminary study showed no change in the free fraction of paclitaxel from 18 to 48 h, indicating that the equilibrium was achieved at ≥18 h. In subsequent experiments, 100-μl aliquots were obtained from donor and receiver compartments at 48 h.

The free fraction of paclitaxel (F_{free}) was calculated from Eq. 5:

$$F_{free} = 1 - \frac{C_b}{C_b + C_f} \quad (5)$$

where C_b is the concentration of the bound paclitaxel in the donor compartment and C_f is the free concentration in the receiver compartment, at equilibrium.

Determination of critical micelle concentration of Cremophor

The method of De Vendittis et al. was used to determine the CMC of Cremophor [5]. Solubilization of the fluorescent probe, i.e. magnesium salt of ANSA, by micelles present in solution results in an increase in the fluorescent signal. Hence, the CMC is the surfactant concentration at which there is an abrupt increase in the fluorescent intensity, i.e. abrupt changes in the slope of the plot of fluorescence intensity versus surfactant concentration. Stock solutions of ANSA and Cremophor were prepared in 50 m*M* HEPES buffer (pH 7.0). To each well of a 96-well plate was added, 50 μl Cremophor (0.001% to 1%), 50 μl HEPES buffer (50 m*M*) and 50 μl ANSA (10 m*M*) and the plate incubated for 1 h at 37 °C. The fluorescent intensity was measured using a fluorescent spectrophotometer, with excitation and emission wavelengths of 390 and 460 nm, respectively.

Data and statistical analysis

The urine and tissue concentration data were analyzed using nonlinear least squares regression by the multivariate secant method and the NLIN procedure of SAS/STAT software (SAS Institute, Cary, N.C.). The two criteria used to evaluate goodness of fits were sum of squared residuals and distribution of residuals. The normality of the pharmacokinetic parameters was tested using SPSS software (Chicago, Ill.). Because these parameters showed a normal distribution, comparisons of pharmacokinetic parameters in different treatment groups were performed using a parametric test, i.e. an unpaired two-tailed Student's *t*-test, assuming equal variance in samples. Comparisons with more than two independent variables were performed using ANOVA.

Results

Urine and plasma pharmacokinetics of paclitaxel

Figure 1 shows the decline in urine concentration of paclitaxel as a function of instillation time. Table 1 summarizes the urine pharmacokinetic parameters. The concentration at zero time is the concentration of paclitaxel in the dosing solution. The drop in urine concentration from zero time to 5 min, when the first sample was obtained, reflects the dilution of paclitaxel by the residual urine in the bladder. As treatment progressed, the ongoing urine production also resulted in

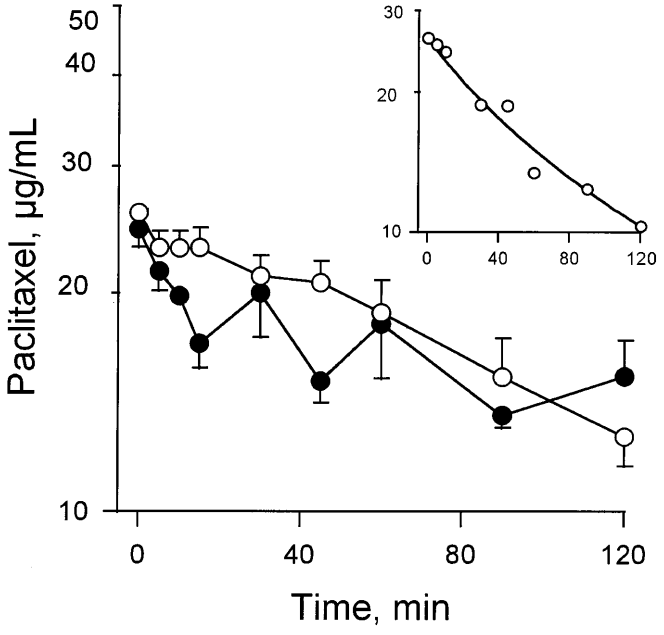

Fig. 1 Urine pharmacokinetics of paclitaxel. Five dogs were given an intravesical dose of 500 µg paclitaxel dissolved in 20 ml 0.22% w/v Cremophor and 0.21% v/v ethanol. The data point at zero time is the concentration in the dosing solution. The later data points represent the measured concentrations. The results of the present study (*open circles*) are shown together with the results of our previous study using water as the solvent (500 µg/20 ml, *closed circles*) [24]. Values are means ± SEM ($n = 5$ in each group). The lines connect the experimental data points and are not computer-fitted. *Inset*: a representative profile in one dog (dog 1); the line is computer-fitted using Eq. 2

Table 1 Bladder tissue pharmacokinetics of paclitaxel. Five dogs received an intravesical dose of 500 µg paclitaxel dissolved in 20 ml of 0.22% w/v Cremophor and 0.21% v/v ethanol solution. Bladders were removed after the 120-min treatment. Each bladder was divided into dome, and left and right lateral sections and the sections were analyzed for drug concentration-depth profiles. Bladder tissue pharmacokinetic parameters were obtained by fitting Eq. 1 to the data. C_{avg} (average tissue concentration) was determined as the area under the tissue concentration-depth profiles divided by the net total depth. C_u is the urine concentration at 120 min. C_{uro} is the concentration in the urothelium. C_b is the concentration in the deepest tissue layer. The results of a previous study in which paclitaxel was dissolved in water are also shown for comparison [23]. Values are the means of 13 tissues for each group. The remaining two tissue sections were contaminated by urine and were discarded

		C_u (µg/ml)	V_{res} (ml)	k_0 (ml/min)	k_a ($min^{-1} \times 10^{-4}$)	C_{uro} (µg/g)	C_{avg} (µg/g)	C_b (µg/g)	C_{uro}:C_u	C_{avg}:C_u	$w_{1/2}$ (µm)
Cremophor/ ethanol	Range	9.80–15.7	0.00–6.31	0.09–0.18	0.00–10.1	0.62–4.24	0.11–1.54	0.00–0.76	0.05–0.46	0.05–0.46	201–768
	Median	12.6	0.00	0.12	1.90	1.63	0.33	0.19	0.16	0.17	328
	Mean ± SD	12.5 ± 2.32	1.36 ± 2.78	0.13 ± 0.04	3.99 ± 4.73	1.87 ± 1.06	0.50 ± 0.41	0.26 ± 0.24	0.16 ± 0.12	0.18 ± 0.09	373 ± 153
Water	Range	11.6–19.9	1.90–5.00	0.04–0.11	6.30–20.0	4.9–10.2	1.4–2.6	0.55–1.8	0.42–0.55	0.10–0.13	262–457
	Median	14.0	2.10	0.07	19.0	7.8	1.5	0.86	0.49	0.12	402
	Mean ± SD	15.4 ± 4.20	3.00 ± 1.70	0.07 ± 0.03	15.0 ± 7.60	7.50 ± 2.50	1.80 ± 0.60	1.00 ± 0.50	0.48 ± 0.06	0.12 ± 0.01	381 ± 78
P-value		0.25	> 0.20	< 0.05	< 0.05	< 0.01	< 0.01	< 0.05	< 0.01	< 0.05	0.47

additional dilution and further reduction in drug concentration. The hybridized first-order rate constant ($k_a + k_d$) is a combination of drug removal by absorption and degradation. Drug degradation was likely insignificant because it has been shown that paclitaxel is stable in urine for 120 min at pH of 5 to 7 [19] and because the major degradation product 7-epitaxol was not detected in urine or in bladder tissue in this study (data not shown). Therefore, the rate constant reflected the rate of drug absorption.

Of the 40 plasma samples collected from five dogs, only one sample showed a detectable level of paclitaxel of 0.63 ng/ml. The low paclitaxel concentration in plasma indicates insignificant systemic bioavailability of intravesical paclitaxel.

Bladder tissue pharmacokinetics of paclitaxel

Figure 2 shows the concentration-depth profile of paclitaxel in bladder tissues. Table 1 summarizes the tissue pharmacokinetic parameters. The drug concentration in the urothelium was about 20% of the concentration in urine collected at the same time. The drug concentrations in tissue declined exponentially as the drug moved through the loosely packed and capillary-perfused connective tissue, reaching an equilibrium with the capillary blood at a concentration of 0.50 μg/g at a depth of ~1500 μm. Mass balance analysis showed that about 92% of the dose was recovered in the 120-min urine samples and about 0.4% in bladder tissues. The three sections of the bladder (dome, and right and left lateral sides) showed comparable concentrations (data not shown).

Urine, plasma and tissue pharmacokinetics of Cremophor

Owing to the limited volume of the urine samples, we measured the Cremophor concentration only in the 120-min sample. The average value was 1225 ± 300 μg/ml or 0.12 ± 0.03% (mean ± SD, range 1252–1527 μg/ml). In the bladder tissue, the highest Cremophor concentration was observed in the urothelium (3.60 ± 3.18 μg/g or 0.00036 ± 0.00031%, range 0.00004–0.0009%), which was about 0.3% of the concentration in urine collected at the same time. The drug concentrations then declined exponentially with respect to tissue depth to the lowest level of 0.10 ± 0.03 μg/g or 0.00001 ± 0.000003% (Figure 3). Over 99% of the administered Cremophor dose was recovered in the

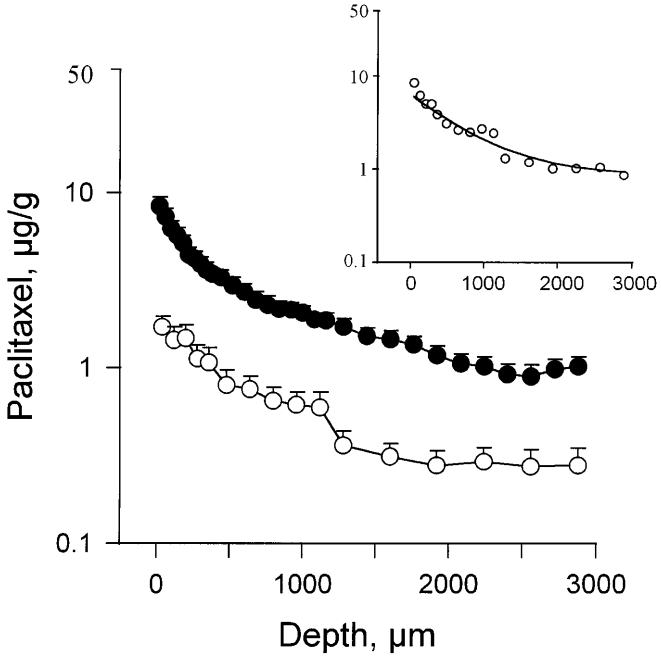

Fig. 2 Paclitaxel concentration in bladder tissue versus depth. Five dogs were given an intravesical dose of 500 μg paclitaxel dissolved in 20 ml 0.22% w/v Cremophor and 0.21% v/v ethanol. The results of the present study (*open circles*) are shown together with the results of our previous study using water as the solvent (500 μg per 20 ml, *closed circles*) [24]. Values are means ± SEM (n = 13 bladder tissues in each group). The remaining two tissue sections were contaminated by urine and were discarded. The lines connect the experimental data points and are not computer-fitted. *Inset*: a representative profile in one tissue section; the line is computer-fitted using Eq. 1

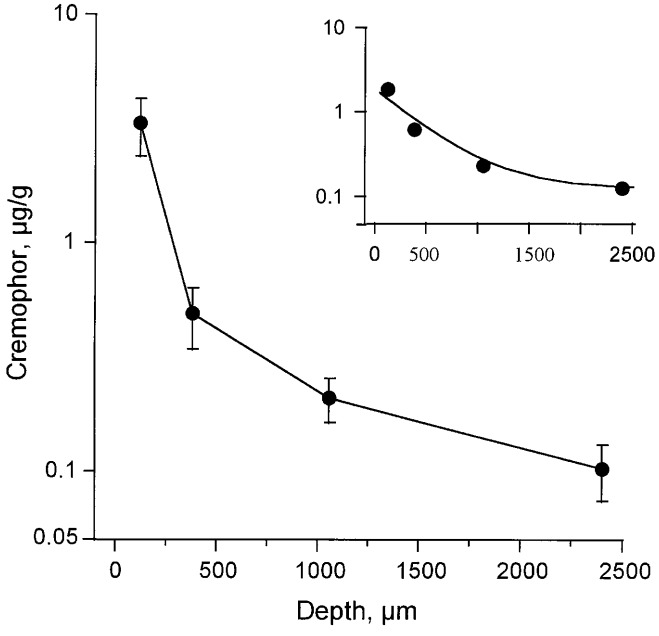

Fig. 3 Cremophor concentration in bladder tissue versus depth. Five dogs were given an intravesical dose of 500 μg paclitaxel dissolved in 20 ml 0.22% w/v Cremophor and 0.21% v/v ethanol. The total dose of Cremophor was 44 mg per 20 ml. Values are means ± SEM (n = 9). The remaining six tissue sections were either contaminated with urine or lost during extraction. The lines connecting the experimental data points and are not computer fitted. *Inset*: a representative profile in one tissue section; the line is computer-fitted using Eq. 1

urine at 120 min and less than 0.001% in the bladder wall, indicating insignificant penetration of Cremophor through the urothelium. The Cremophor concentrations in plasma were below the detection limit of 0.005% [26].

Effect of Cremophor/ethanol on paclitaxel pharmacokinetics

Table 1 shows the results of the present study in which paclitaxel was dissolved in Cremophor/ethanol and, for comparison, the results of our previous study in which paclitaxel was dissolved in water. The comparison reveals the following. (A) The addition of Cremophor/ethanol reduced the urothelial concentration, the urothelial-to-urine concentration ratio and the average tissue concentration, each by ~75%. This indicates that Cremophor/ethanol decreased the paclitaxel partitioning across the urothelium. (B) The addition of Cremophor/ethanol did not alter the rate of paclitaxel concentration decline in the tissue (i.e. no change in $w_{1/2}$). (C) Cremophor/ethanol nearly doubled the urine production rate but otherwise had no effect on the urine pharmacokinetics of paclitaxel. (D) Cremophor/ethanol decreased the first-order absorption rate constant by 70%. (E) Cremophor/ethanol had no effect on the plasma pharmacokinetics of paclitaxel; both groups showed insignificant or undetectable plasma concentrations (<0.4 ng/ml).

Effect of Cremophor on the free fraction of paclitaxel

Figure 4 shows that the CMC of Cremophor was 0.008%. This value is comparable to previously reported values [7, 13]. Figure 5 shows the results of equilibrium dialysis. For paclitaxel dissolved in PBS, the paclitaxel concentrations in the receiver compartment after 48 h equilibration were not significantly different from those in the donor compartment, confirming the attainment of equilibrium. The free fraction was calculated to be 94 ± 2.8%. The addition of ethanol at concentrations ranging from 0.0025% to 1% did not alter the paclitaxel concentrations in the donor and receiver compartments. This was as expected because ethanol could freely diffuse across the dialysis membrane and therefore would not alter the equilibrium. Cremophor had no significant effect at concentrations below 0.01% (i.e. below the CMC). However, at concentrations between 0.01% and 1%, Cremophor, with or without ethanol, resulted in a significant concentration-dependent reduction in the diffusible paclitaxel. At concentrations of 0.0625% and 0.25%, which are within the range of the Cremophor concentrations in urine, the free fraction was reduced to 46% and 18%, respectively. These results indicate that Cremophor, at concentrations above the CMC, caused sequestration of paclitaxel in Cremophor micelles and thereby reduced the free fraction of paclitaxel.

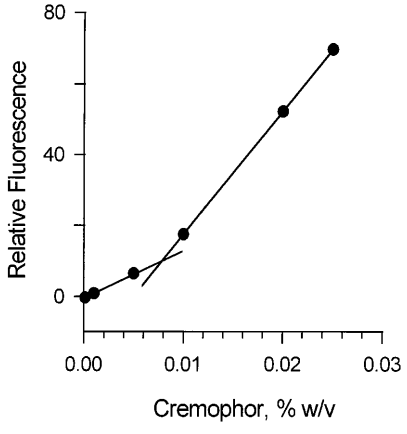

Fig. 4 Determination of critical micelle concentration of Cremophor. The fluorescence intensity of ANSA increased with Cremophor concentration. Data are corrected for the baseline fluorescence intensity of the ANSA solution without Cremophor. The Cremophor concentration where the two extrapolated lines intersect corresponds to the CMC

Discussion

Our results indicate that the Cremophor concentrations in urine during the 120-min treatment were sufficient to form micelles and resulted in sequestration of paclitaxel into or onto the Cremophor micelles. This in turn reduced the free fraction and partitioning of paclitaxel across the bladder urothelium. There was no difference in the drug concentration in the final urine sample between the water and the Cremophor/ethanol groups, because the 70% decrease in the drug absorption rate was offset by the 85% increase in urine production rate. The reasons for the increase in urine production rate in the Cremophor/ethanol group are not apparent.

The entrapment of paclitaxel in Cremophor micelles may lead to substantial intersubject variability, for the following reasons. During intravesical treatment, which is usually administered over 2 h, urine is constantly being produced. We have shown that the urine production in human patients, during a 120-min intravesical treatment, ranges from 10 to 648 ml [2, 4]. This wide range of urine volume will alter the Cremophor concentration. For example, the Cremophor concentration in the clinical formulation is 0.22%, which is 20-fold higher than the CMC of cremophor. A 25-fold dilution of the 20 ml dosing solution by 480 ml newly produced urine would reduce the Cremophor concentration from 0.22% in the formulation to below the CMC of 0.01%. Consequently, the free fraction of paclitaxel would increase from ~25% to ~100%, resulting in fluctuating drug penetration into the bladder. Second, the stability of micelles is altered in the presence of salts or organic materials. For example, urea

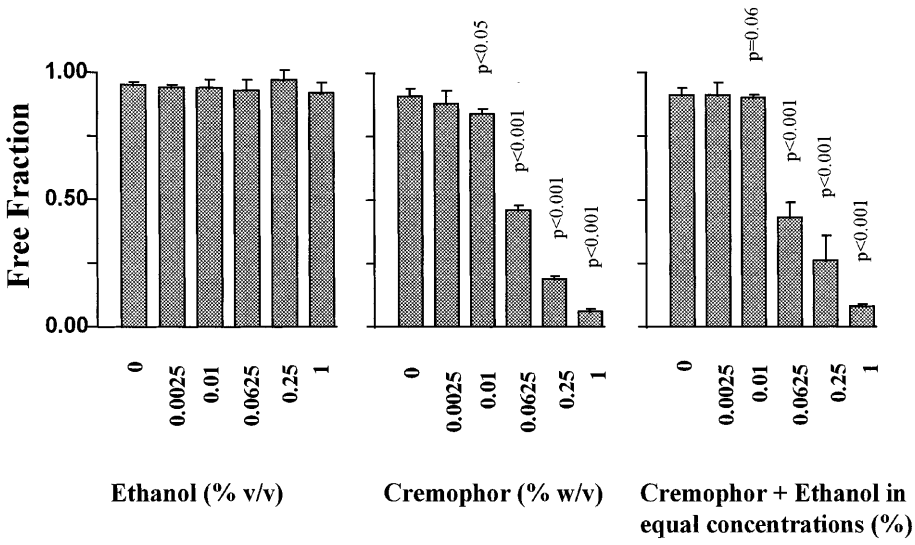

Fig. 5 Effect of Cremophor and ethanol on the free fraction of paclitaxel. The free fraction of paclitaxel in solution containing Cremophor and/or ethanol was determined using equilibrium dialysis and calculated using Eq. 5. The free fractions that are significantly different from the control values (PBS alone without ethanol or Cremophor) are indicated. Values are means ± SD ($n = 3$)

has been shown to alter the CMC of ionic and nonionic surfactants [16].

Another factor that needs to be considered is the pharmacological activities of Cremophor. Cremophor, at a concentration of ~0.1%, induces G1 phase arrest and reverses the paclitaxel resistance mediated by the overexpression of the mdr*1* p-glycoprotein (PGP) [3, 6, 14, 18, 28]. A comparison of this pharmacologically active concentration with the Cremophor concentrations in urine and bladder tissues indicates that the concentration in the urine but not the concentration in the bladder tissues is sufficient to reverse the PGP-mediated resistance to paclitaxel. Accordingly, the Cremophor in the FDA-approved formulation may affect the activity of paclitaxel in tumors that are either located on the surface of the urothelium or protruding, but not in tumors located in the bladder wall.

When dissolved in Cremophor/ethanol, the plasma concentrations of paclitaxel during the 120-min intravesical treatment were below the assay detection limit of 0.3 ng/ml, and are therefore >46 000-fold lower than the urine concentration of 14 μg/ml, >1600-fold lower than the bladder tissue concentration of 0.5 μg/g, and >16 000-fold lower than the plasma concentration of 5.0 μg/ml attained during an intravenous systemic therapy of 175 mg/m^2 over 3 h [12]. Hence, we conclude that intravesical administration of paclitaxel in Cremophor/ethanol will provide a significant targeting advantage for tumors located in the bladder and will produce less systemic host toxicity compared to intravenous therapy, although the targeting advantage is likely to be lower and more variable than when water is used as the solvent.

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