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ThioTEPA pharmacokinetics during intravesical chemotherapy and the influence of Tween 80

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Summary. A pharmacokinetic study of randomised cross-over design was carried out in which eight patients with recurrent stage pTa or pT1 transitional cell carcinoma of the bladder were given thioTEPA (30 mg) in distilled water or in 10% (v/v) Tween 80 (30 ml) intravesically for 2 h, followed 3 months later by the alternative treatment. ThioTEPA and its primary metabolite, TEPA, were measured in plasma and urine using a sensitive and specific chromatographic assay. Large differences between patients were observed in the proportion of thioTEPA absorbed, ranging from 20%–78%. Peak plasma levels of thioTEPA were observed within 1 h of intravesical administration. By 2 h after administration the plasma levels of TEPA were similar to those of thioTEPA and, in contrast to those of the parent compound, remained at a similar level over the next 4 h. The rate of absorption of thioTEPA was not influenced by Tween 80, but it did cause statistically significant increases in mean peak plasma levels (from 101 to 154 ng/ml) and mean AUC values (from 0.376 to 0.496 μg h per ml) and a decrease in the mean half-life (from 1.83 to 1.25 h). To obtain plasma levels similar to those achieved after instillation with thioTEPA alone, the dose should be reduced with Tween 80.

Introduction

ThioTEPA was the first drug shown to be effective for the treatment of superficial bladder cancer following intravesical administration [17]. Although thioTEPA continues to be the agent most frequently used for intravesical chemotherapy, it has the disadvantage that systemic uptake can occur, resulting in sporadic and unpredictable myelosuppression. Although this rarely interferes with treatment today, in early studies using high doses some patients died as a result of myelosuppression [26].

There are disparities in the literature concerning the decomposition and metabolism of thioTEPA and its degree of absorption from the bladder [4]. The inconsistent pharmacological and pharmacokinetic data result in part from the absence of a reliable method for measuring thioTEPA and its metabolites. However, a sensitive and specific assay for thioTEPA and its major metabolite (TEPA) is now available [19]. The primary purpose of this study was to use this assay to study thioTEPA pharmacokinetics during intravesical chemotherapy. A detailed understanding of the behaviour of thioTEPA following its instillation into the bladder could lead to safer and more effective chemotherapy.

Tween 80 is a non-ionic detergent widely used in the food and pharmaceutical industries. In combination with chemotherapeutic drugs, it can enhance drug activity in vitro, in experimental animals and in patients. Drug cytotoxicity is increased by Tween 80 in vitro [16, 24] and in experimental tumours in animals [3, 25]. The combination of doxorubicin with 10% Tween 80 for intravesical chemotherapy overcame drug resistance in three of six patients [8]. We have shown that the addition of Tween 80 to each of the four drugs most frequently used for intravesical chemotherapy (doxorubicin, Epodyl, mitomycin C, thioTEPA) enhanced their cytotoxicities against a human bladder cancer cell line in vitro [22]. Thus, thioTEPA given in combination with Tween 80 might be more effective than the drug alone for the treatment of superficial bladder cancer. However, there is a risk that Tween 80 would increase systemic uptake and, consequently, the toxicity of thioTEPA. Therefore, the secondary purpose of this study was to investigate the influence of Tween 80 on thioTEPA uptake and disposition.

Patients and methods

Patients. This study was approved by the University Ethical Committee and included eight patients (one woman and seven men) with stage pTa or pT1 superficial transitional cell carcinoma of the bladder. The mean age of the patients was 68 years (range, 64–79 years). The presence of active disease at the last two review cystoscopies was confirmed by cystourethroscopy and histopathology. Patients were excluded from the study if any of the following criteria was fulfilled: WBC count of $<4{,}000/\mu l$; platelet count of $<100{,}000/ml$; haemoglobin concentration of <10 g/dl; bladder capacity of <100 ml; presence of urinary extravasation or gross reflux; pregnancy; urinary infection; prior therapy of the disease other than endoscopic surgery during the 12 months before this study.

Dosing and sampling schedule. Intravesical chemotherapy was given between 1 and 40 days after surgical resection of

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Table 1. Comparative pharmacokinetic parameters of thioTEPA alone and in combination with Tween 80 in plasma

	Ka (h^{-1})	$t_{1/2}$ (h)	AUC^{∞} ($\mu g \cdot h \cdot ml^{-1}$)	C_{max} ($\mu g \cdot ml^{-1}$)	t_{max} (h)
ThioTEPA	2.15 ± 0.24	1.83 ± 0.33	0.376 ± 0.106	0.101 ± 0.014	0.962 ± 0.057
ThioTEPA + Tween 80	2.18 ± 0.52	1.25 ± 0.14	0.496 ± 0.121	0.154 ± 0.081	0.960 ± 0.132
P value	NS	<0.05	<0.05	<0.05	NS

Data represent the means ± SE ($n = 8$); NS, not significant

Table 2. Pharmacokinetic parameters of TEPA in plasma after intravesical administration of thioTEPA alone and in combination with Tween 80

	AUC (to 6 h) ($\mu g \cdot h \cdot ml^{-1}$)	C_{max} ($\mu g \cdot ml^{-1}$)	t_{max} (h)
ThioTEPA	0.12 ± 0.03	0.033 ± 0.008	4.67 ± 0.67
ThioTEPA + Tween 80	0.18 ± 0.07	0.038 ± 0.012	3.62 ± 0.96

Data represent the means ± SE ($n = 6$)

all tumour apparent during cystourethroscopy. Each patient received two courses of therapy, each at the same interval following cystourethroscopy. One consisted of thioTEPA alone and one comprised thioTEPA combined with Tween 80; the courses were separated by a period of approximately 3 months. The study was of a randomised, cross-over design to negate period effects, and half of the patients received thioTEPA alone first.

The patients had no food or fluid intake from 2200 hours on the night prior to treatment until 2 h after the instillation of the drug. Immediately prior to treatment an indwelling cannula was inserted into a forearm vein. The bladder was catheterised and the urine collected, and 30 mg thioTEPA (Lederle Laboratories, Gosport, UK) dissolved in 30 ml distilled water alone or in 30 ml 10% (by vol.) Tween 80 (provided by Dr. Stephan Eksborg, Karolinska Pharmacy, Stockholm, Sweden) in distilled water was instilled into the bladder for 2 h. A sample of blood (5 ml) was drawn at 0, 0.25, 0.5, 0.75, 1, 2, 4, 6 and 24 h after instillation and transferred to a heparinised

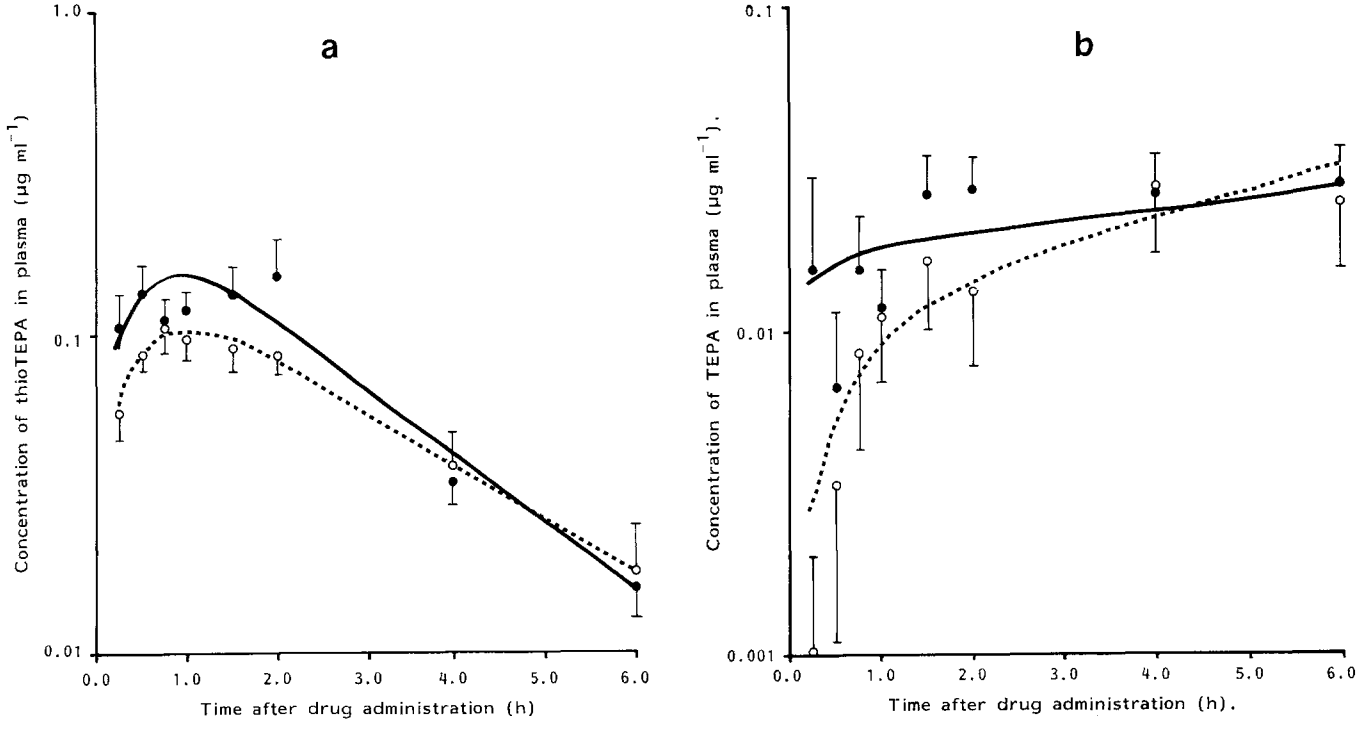

Fig. 1. Semilogarithmic fitted plots of plasma concentrations of **a** thioTEPA and **b** TEPA (means ± SE) after administration of the drug alone (*open symbols*) or with Tween 80 (*closed symbols*)

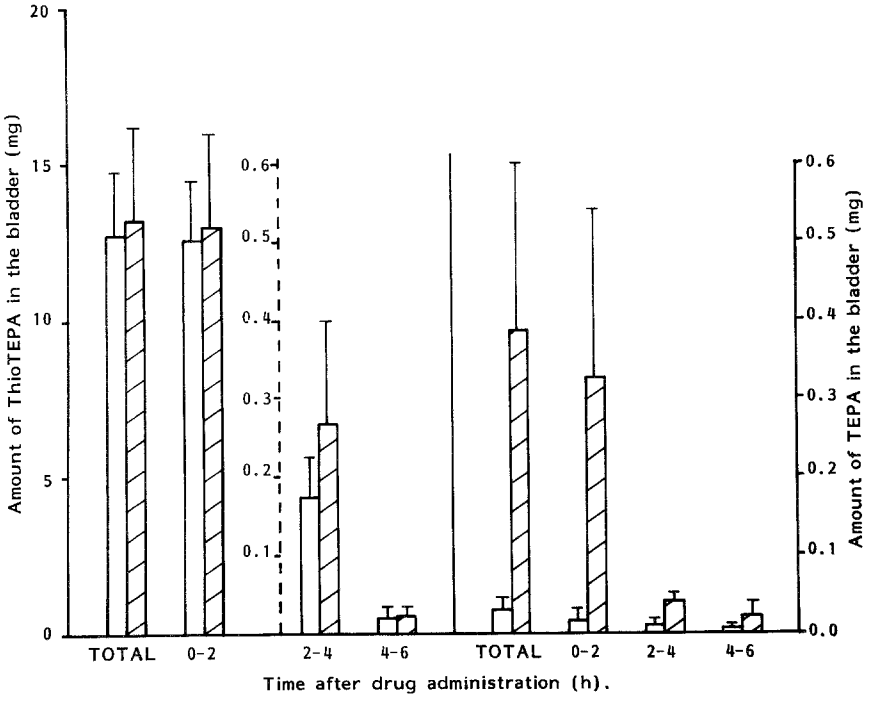

Fig. 2. Amounts of thioTEPA and TEPA in bladder fluid (means +SE) during 2 h periods and their total, after administration of the drug alone (*open bars*) or with Tween 80 (*hatched bars*)

tube. Plasma was separated and stored at −80° C immediately. The fluid in the bladder was collected at 2, 4 and 6 h after instillation; each volume was recorded and the pH and osmolality were determined. An aliquot (10 ml) of each of these specimens was stored immediately at −80° C for analysis of the drug and its metabolites. Additional specimens of blood were taken at 14 and 21 days after treatment for haematological analysis.

Analytical methods. ThioTEPA and its primary metabolite, TEPA, were measured as previously described [19]. Briefly, after extraction of the drug and metabolite from plasma or urine using Sep-Pak C_{18} cartridges, the compounds were separated by capillary chromatography, detected using a nitrogen detector and quantified by reference to an internal standard, hexaethylphosphoramide. The limits of sensitivity were 1–5 ng/ml. Mean analytical recoveries from a concentration of 50 ng/ml were 74% and 94% and inter-assay (n = 12) variations (standard errors) were 7.0% and 8.0% for TEPA and thioTEPA, respectively.

Data analysis. Plasma concentration vs time curves for

Table 3. ThioTEPA levels in individual patients treated with thioTEPA or thioTEPA in combination with Tween 80, showing the duration of the period between surgical resection and chemotherapy

Treatment order	Treatment	C_{max} ($\mu g \cdot ml^{-1}$)	AUC^{∞} ($\mu g \cdot h \cdot ml^{-1}$)	Period between cystourethroscopy and chemotherapy (days)
2	ThioTEPA + Tween 80	0.211	1.246	35
1	ThioTEPA alone	0.115	1.046	35
1	ThioTEPA + Tween 80	0.091	0.311	35
2	ThioTEPA alone	0.060	0.168	35
2	ThioTEPA + Tween 80	0.161	0.350	1
1	ThioTEPA alone	0.175	0.432	1
1	ThioTEPA + Tween 80	0.120	0.083	19
2	ThioTEPA alone	0.105	0.136	20
2	ThioTEPA + Tween 80	0.048	0.485	2
1	ThioTEPA alone	0.058	0.369	2
2	ThioTEPA + Tween 80	0.153	0.618	6
1	ThioTEPA alone	0.089	0.470	13
1	ThioTEPA + Tween 80	0.316	0.475	23
2	ThioTEPA alone	0.138	0.196	40
1	ThioTEPA + Tween 80	0.135	0.399	15
2	ThioTEPA alone	0.072	0.196	8

Table 4. ThioTEPA and TEPA levels in the bladder contents at the end of the instillation period (at 2 h) and in the fluid collected 2 (at 4 h) and 4 h (at 6 h) later

	2 h	4 h	6 h
ThioTEPA alone:			
ThioTEPA (mg)	12.57 ± 1.99 (6.64 – 24.09)	0.174 ± 0.054 (0 – 0.515)	0.018 ± 0.018 (0 – 0.056)
TEPA (mg)	0.016 ± 0.016 (0 – 0.096)	0.010 ± 0.006 (0 – 0.031)	0.0033 ± 0.0024 (0 – 0.015)
ThioTEPA + Tween 80:			
ThioTEPA (mg)	13.05 ± 2.97 (4.82 – 27.71)	0.267 ± 0.137 (0.040 – 1.180)	0.021 ± 0.013 (0.001 – 0.106)
TEPA (mg)	0.327 ± 0.215 (0 – 1.312)	0.039 ± 0.011 (0 – 0.066)	0.021 ± 0.021 (0 – 0.127)

Data represent the means ± SE (thioTEPA, $n = 8$; TEPA, $n = 6$). The numbers in parentheses indicate the range of values

Table 5. Estimates of the volume of distribution and total body clearance (as ratios with bioavailability) of thioTEPA and the effect of Tween 80

	V/F (l)	Cl/F ($l \cdot h^{-1}$)
ThioTEPA alone	208.7 ± 18.9	103.2 ± 23.6
ThioTEPA + Tween 80	126.9 ± 16.7	94.6 ± 30.4
P value	< 0.05	NS

Data represent the means ± SE ($n = 8$); NS, not significant

Table 6. Haematology of patients before and at 14 and 21 days after intravesical chemotherapy

	ThioTEPA	ThioTEPA + Tween 80
WBC ($\times 10^9$/l):		
Before	7.9 ± 0.6	8.7 ± 0.3
14 days	8.2 ± 0.6	8.0 ± 0.4
21 days	7.2 ± 0.5	8.0 ± 0.7
Platelets ($\times 10^9$/l):		
Before	292 ± 37	275 ± 33
14 days	292 ± 25	295 ± 31
21 days	253 ± 36	267 ± 45
Haemoglobin (g/dl):		
Before	14.3 ± 0.6	14.6 ± 0.5
14 days	14.3 ± 0.3	14.0 ± 0.3
21 days	14.2 ± 0.4	14.0 ± 0.3

Data represent the means ± SE ($n = 8$)

thioTEPA were analysed using an extended least-squares modelling programme, MKMODEL (Elsevier-Biosoft, Cambridge, UK). Data were fitted to a one-compartment model with first-order input. The experimental constants obtained included the rate constant of absorption (K_a), that of elimination (K) and DF/V, where D is the dose, F is the fraction absorbed and V is the volume of distribution. Using these values, the following parameters were calculated by standard methods [9]: half-life ($t_{1/2}$), maximal concentration in plasma (C_{max}) and the time taken to reach C_{max} (t_{max}). The programme included calculation of the AUC using a combination of the linear and logarithmic trapezoidal rules [9]. The value of AUC to infinity (AUC^{∞}) was estimated by addition of the residual area, given by C^*/K, where C^* is the final measured concentration.

The AUC values for TEPA were calculated to 6 h and values of C_{max} and t_{max} were determined by inspection of the individual profiles. All pharmacokinetic data were assessed using non-parametric statistics. Comparisons relating to the influence of Tween 80 were analysed using the Wilcoxon signed rank test [14].

Results

All samples were collected, with the exception of the two pre-instillation urine specimens from one patient whose bladder was empty on both occasions. Determinations of TEPA in samples from two patients were lost during the analytical process; therefore, the data for the metabolite are based on the results from the other six patients.

ThioTEPA pharmacokinetics

The pharmacokinetic parameters in plasma are summarised for thioTEPA in Table 1 and Fig. 1 a and for TEPA in Table 2 and Fig. 1 b. Peak plasma levels of thioTEPA were recorded approximately 1 h after intravesical administration, with a maximal concentration of approximately 100 ng/ml. The half-life of the drug in plasma was 1.83 h. TEPA began to appear in plasma shortly after thioTEPA, and measurable levels were still present 5 h after the drug had been instilled. In contrast to thioTEPA, levels of TEPA remained relatively constant between 1 and 6 h after drug administration. There did not appear to be a relationship between thioTEPA levels in plasma and the period between cystourethroscopy and chemotherapy (see Table 3).

The levels of thioTEPA in the bladder at the end of the instillation period and at 2 and 4 h after treatment are

Table 7. Osmolality and pH values of urine before (0), at the completion of (2 h) and 2 and 4 h (4 h, 6 h) after intravesical chemotherapy

	ThioTEPA	ThioTEPA + Tween 80
pH:		
0	6.0 ± 0.2	6.2 ± 0.2
2 h	6.6 ± 0.2	6.6 ± 0.2
4 h	5.9 ± 0.3	6.0 ± 0.2
6 h	5.6 ± 0.2	6.1 ± 0.2
Osmolality (mOsmol/kg):		
0	616 + 68	643 + 76
2 h	553 + 42	581 + 49
4 h	645 + 58	674 + 65
6 h	655 + 58	719 + 60

Data represent the means ± SE ($n = 8$)

summarised in Table 4 and Fig. 2. On completion of treatment between 6.6 and 24.1 mg thioTEPA was recovered, indicating that between 20% and 78% of the drug had been absorbed or degraded, excluding that which had been excreted back into the bladder. Only small quantities of thioTEPA were recovered 2 and 4 h later in the urine. Minute amounts of TEPA were found in the bladder contents, the highest concentration being detected at the end of the instillation period.

Influence of Tween 80 on thioTEPA pharmacokinetics

The urinary levels of thioTEPA and its primary metabolite following co-administration with Tween 80 are summarised in Table 4 and Fig. 2. Whereas levels of thioTEPA in the bladder were not significantly different in the presence of Tween 80, TEPA levels were, on average, higher at each time point.

In the plasma, Tween 80 did not appear to alter the rate of absorption (see Table 1). Neither K_a (the rate constant of absorption) nor t_{max} (the time at which the maximal concentration was recorded) changed significantly. However, the AUC was significantly increased in the presence of Tween 80, and the half-life of the drug in plasma was significantly reduced. As there was no significant increase in the clearance of the drug, the reduced half-life was due to a significant decrease in the volume of distribution (see Table 5). The increased levels of thioTEPA were also reflected in a 50% greater AUC for TEPA (see Table 2).

Haematology

There was no clinical evidence of systemic toxicity as a result of treatment, with or without Tween 80. There were no clinical manifestations of myelosuppression, and in no case did the WBC or platelet count or haemoglobin concentrations fall below normal levels (see Table 6). The only statistically significant change in patients treated with the combination of drug plus Tween 80 was a fall in the WBC count from the pre-treatment levels, observed at 14 days after instillation. This may reflect the higher AUC in plasma in the presence of Tween 80.

pH and osmolality

The pH and osmolality data are summarised in Table 7. The pH was higher at 2 h (mean, 6.6) than before or after treatment (approximate value, 6.0), reflecting the higher pH (8.2) of the instillate. Similarly, at 2 h the osmolality was reduced by the instillate compared with the pre- and post-treatment levels. There were no clear associations between either pH or osmolality values and the pharmacokinetic parameters; however, the number of cases examined was small.

Discussion

ThioTEPA is the drug most frequently used for intravesical chemotherapy of bladder cancer. In some early studies fatal myelosuppression occurred as a result of systemic absorption. Fatalities are no longer reported, although approximately 20% of patients experience a decrease in WBC or platelet count below normal values [26]. This study describes the first systematic pharmacokinetic analysis of thioTEPA pharmacokinetics during intravesical chemotherapy in patients, using a sensitive and specific direct assay of the drug and its primary metabolite, TEPA.

Peak plasma levels of thioTEPA occurred within 1 h of administration and then fell, with a mean half-life of 1.25 h in the presence of Tween 80 and the 1.83 h for the drug alone. These values are not directly comparable with those obtained following systemic drug administration, because during intravesical chemotherapy the drug continues to be available for absorption from the bladder throughout the instillation period, in the present study for 2 h. Consequently, the drug can be constantly recycled, and excretion rates will be underestimated until the instillate is drained. Nevertheless, the pharmacokinetic parameters calculated on the basis of these data are in general agreement with previous studies on systemic administration in man [5, 19, 21] and experimental animals [1, 7, 20, 27].

Plasma levels of thioTEPA peaked within 1 h of administration; thus, systemic toxicity will not increase proportionately with exposure periods of >1 h. The proportion of the parent drug disappearing from the bladder during the 2 h exposure period ranged from 20% to 78%, most of which was absorbed systemically. The number of cases studied was too small to ascertain whether factors such as recent surgery, tumour size or pH or other factors might have influenced systemic uptake. However, these factors have been indirectly examined in previous studies by measuring the degree of myelosuppression. As expected, high dose rates and frequent instillations are more likely to result in myelosuppression, but treatment within 48 h of surgery is not a risk factor if the weight of the drug instilled is $\leqslant 60$ mg [26].

The primary metabolite, TEPA, is thought to provide a substantial contribution to toxicity and antitumour activity following systemic administration, and the plasma levels of TEPA were similar to those of thioTEPA within 2 h of systemic administration [5]. TEPA was eliminated from the plasma at a much lower rate than thioTEPA, in agreement with previous studies in diverse species [19]. In the presence of Tween 80, considerably more TEPA was present in the bladder. This could reflect increased excretion of TEPA, but, as the pharmacokinetic data do not support this hypothesis, it seems more likely that

thioTEPA is degraded more rapidly in the presence of Tween 80. Subsequent metabolites exist, particularly alkylating species, which could contribute to toxicity and are not assessed by the analytical method used. There are as yet unidentified peaks on the gas-liquid chromatographic (GLC) analysis of thioTEPA metabolites (McDermott, unpublished data).

At least four studies measuring thioTEPA following intravesical administration have been published. One of these measured the amount of thioTEPA recoverable from the bladder during treatment and, on this basis, calculated that up to 97.3% of the drug was absorbed [23]. In the first study of thioTEPA, it was calculated that approximately one-third of the drug was absorbed [17]. A later study measured alkylating activity in the plasma at hourly intervals for 5 h following administration [18]. Peak levels were observed at approximately 1 h, as confirmed in the present study, but alkylating activity remained stable over the rest of the observation period. Similarly, following intravesical administration of ^{32}P-labelled thioTEPA, plasma levels of radioactivity remained relatively high over a 24 h period following intravesical administration [15], in contrast to the present data. These results probably reflect technical differences between the direct assay of thioTEPA [19] and the less sensitive, indirect assays used in earlier studies, which also measured metabolites and other degradation products that may remain in plasma for longer periods than the parent compound.

Tween 80 has been shown to enhance anticancer activity, possibly by increasing drug uptake into tumour cells. In contrast, the systemic absorption of *N*-alkyl carbamates from rat bladders was reduced 76% by the addition of 5% Tween 80 [2]. In the present study, the addition of Tween 80 resulted in a 50% increase in the total systemic dose rate (AUC) and in the maximal plasma concentration (C_{max}). Since there was essentially no apparent change in clearance and $t_{1/2}$ was decreased considerably, the addition of Tween 80 must have significantly reduced the volume of distribution. The kinetic picture suggests that Tween 80 displaces the drug from tissue binding sites. This results in an increase in peak concentration and AUC, partly because the volume of distribution is decreased, and partly because availability is increased. Similar effects could result from an influence of Tween 80 on the distribution of the drug between plasma and red blood cells. Our observation [19] that there is little binding of thioTEPA to serum proteins has recently been confirmed [12]. Plasma levels of doxorubicin have also been increased 4- to 5-fold by Tween 80 following the intravesical administration of the combination in rats [13]. In contrast, when given systemically with Adriamycin to cancer patients, Tween 80 increased the volume of distribution by 3-fold, decreased the AUC by up to 2-fold and increased clearance by the same amount [6].

The pH of the bladder fluid at the end of the 2 h instillation period was significantly raised, reflecting the alkaline pH of the thioTEPA solution, but there were no other significant differences in pH or osmolality. Both of these factors can influence cytotoxicity [10, 11] and could modify the systemic uptake of thioTEPA, but our data were insufficient to determine whether either pH or osmolality had any influence on plasma levels. The increased systemic levels of thioTEPA in the presence of Tween 80 were reflected in a statistically significant decrease in WBC count at 14 days, although the values remained within normal levels. These data indicate that in the presence of 10% Tween 80, the systemic dose increased by approximately 50%.

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