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ThioTEPA pharmacokinetics during intravesical chemotherapy: the influence of dose and volume of instillate on systemic uptake and dose rate to the tumour

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Abstract ThioTEPA is given intravesically in a variety of schedules to treat superficial bladder cancer. In this study, the influence of the dose of ThioTEPA and the volume of instillate on the dose rate to the tumour and the systemic uptake of ThioTEPA was investigated in eight patients with pTa or pTl disease. Each patient received four courses of ThioTEPA consisting of 30 mg of drug/30 ml of distilled water, 30 mg/60 ml, 60 mg/30 ml and 60 mg/60 ml. Blood and urine samples were obtained for 8 h following instillation, and ThioTEPA and TEPA levels were measured. The AUC_{∞} values (areas under the concentration-time curve, extrapolated to infinity) in plasma were approximately 2 factors higher at the two 60-mg doses. However, the AUC value in the bladder was nearly 70% higher when 60 mg of drug was instilled in 30 ml of distilled water as compared with 60 mg in 60 ml. Thus, by decreasing the volume of instillate it is possible to increase the dose rate to the tumour without increasing the systemic toxicity.

Key words ThioTEPA · Intravesical chemotherapy · Dose rate

Introduction

ThioTEPA was the first drug shown to be effective for the treatment of superficial bladder cancer when given intravesically [12], and it continues to be widely used. Unlike the other drugs used to treat superficial bladder cancer, such as Adriamycin and mitomycin C, ThioTEPA has the disadvantage that it readily passes through the bladder [5, 13], causing myelosuppression in up to 20% of patients [18]. It is not possible to predict which patients will suffer these side-effects.

Optimal schedules for administration of ThioTEPA to bladder tumours have not been established. At most centres, between 30 and 60 mg of ThioTEPA is given in volumes ranging from 20 to 100 ml [2, 11], resulting in drug concentrations in the range of 0.5–2.0 mg ml^{-1}. The exposure period usually varies between 30 min and 2 h, producing a wide range of dose rates to individual patients. The number of courses of treatment also vary, ranging from a single administration [2, 17] to multiple instillations over a 12-month period. The single or short courses of therapy tend to be used as an adjuvant to surgery to delay recurrence, whereas more intensive regimes are designed to ablate tumours that are not amenable to surgery.

This study was undertaken to determine the influence of the dose and volume of the instillate on (a) the dose rate to the tumour in the bladder and (b) the amount of ThioTEPA entering the circulation from the bladder. By increasing the concentration of the drug in the bladder, it was possible to increase the dose rate to the tumour without incurring any significant increase in systemic uptake.

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Patients and methods

Patients

This study was approved by the St. Peters' Hospital Ethical Committee and included subjects with recurrent pTa pT1 bladder cancers. The presence of recurrent tumour was determined at check cystoscopy and confirmed by histopathology. Patients were excluded from the study if any of the following criteria were fulfilled: a WBC of $< 4000/mm^3$; platelet count of $< 100{,}000/mm^3$; a

haemoglobin value of $< 10\ g\,dl^{-1}$; a bladder capacity of < 100 ml; and the presence of urinary extravasation or gross reflux, pregnancy, urinary infection or previous therapy other than endoscopic surgery during the 12 months prior to the study.

Dosing and sampling schedule

Intravesical chemotherapy was given after transurethral surgical resection of all tumour, and each patient received four courses of ThioTEPA consisting of 30 mg of drug in 30 ml of distilled water, 30 mg in 60 ml, 60 mg in 30 ml and 60 mg in 60 ml, the courses being given in random order.

The patients had restricted food and fluid intake from 22.00 hours on the evening before treatment until 2 h after instillation of the drug. Immediately before the therapy an indwelling catheter was inserted into a forearm vein. The bladder was catheterised and the urine collected. ThioTEPA (Lederle Laboratories, Gosport, UK) dissolved in distilled water was instilled into the bladder and retained for 2 h. A sample of blood (5 ml) was taken at 0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0 h after instillation and transferred to a heparinised tube. Plasma was separated immediately and two 1-ml aliquots were stored at $-70°C$. The fluid in the bladder was collected at 0, 2, 4, 6 and 8 h, the volume was recorded and two 5-ml aliquots were stored at $-70°C$. Specimens of blood were also obtained for haematological analysis.

Analytical methods

ThioTEPA and TEPA were measured as previously described [14]. After extraction of the drug and metabolite from plasma or urine using Sep-Pak C_{18} cartridges, they 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 $ng\,ml^{-1}$. Mean analytical recoveries of TEPA and ThioTEPA from a concentration of $50\ ng\,ml^{-1}$ were 74% and 94%, respectively, and the interassay ($n = 12$) variations (standard errors) were 7.0% and 8.0%, respectively.

Data analysis

Plasma concentration versus time curves for ThioTEPA were analysed using an extended least-squares modeling programme, MKMODEL (Elsevier-Biosoft, Cambridge, UK). Data were fitted to a one-compartment model with first-order input including a lag phase. The experimental constants obtained included the rate constants of absorption (K_a) and elimination (K) and DF/V, were 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 [8]: the half-life ($t_{1/2}$), apparent clearance (CL/F), maximal concentration in plasma (C_{max}) and time taken to reach C_{max} (t_{max}). The programme included calculation of the area under the concentration-time curve (AUC) using a combination of the linear and logarithmic trapezoidal rules [8]. The value of AUC extrapolated to infinity (AUC_∞) in plasma was estimated by addition of the residual area, given by C^*/K, were C^* is the final measured concentration. Exposure of bladder to the drug (AUC in bladder 0–2 h) was estimated assuming a monoexponential decline in concentration during the instillation period.

Statistical analysis

Normal probability plots were constructed to test the assumption of normality of the data and to identify outlying points. Up to two cases were discarded in the multivariate analysis, which was performed using SPSS-PC software (SPSS Inc., Chicago, USA). Differences with $P \leq 0.05$ were regarded as significant for within-group (dose or volume) effects. In such instances, Student's t-test for paired data was applied to establish differences between individual groups. Values of $P \leq 0.05$ were regarded as significant.

Results

The characteristics of the eight patients, all men, who took part in this study are given in Table 1. The period between the surgery and the initial administration of chemotherapy ranged between 38 and 57 days. There were intervals of 14–61 days between courses of chemotherapy. The time courses of the plasma concentration of ThioTEPA after administration of 30 and 60 mg of drug in either 30 or 60 ml of distilled water are shown as semilogarithmic plots in Fig. 1. Plasma levels were detectable for up to 6 h after administration in the majority of cases. Limited data were obtained after 7 h,

Table 1 Age of the 8 men recruited to this study as well as their bladder-cancer history and the stage and grade of their tumours

Age (years)	Bladder-cancer history (months)	Tumour stage	Tumour grade
73	22	Pa	1
76	35	Pa	2
62	76	Pa	1
49	27	Pa	1
53	8	Pa	1
72	5	P1a	2
64	263	P1b	2
69	334	Pa	1

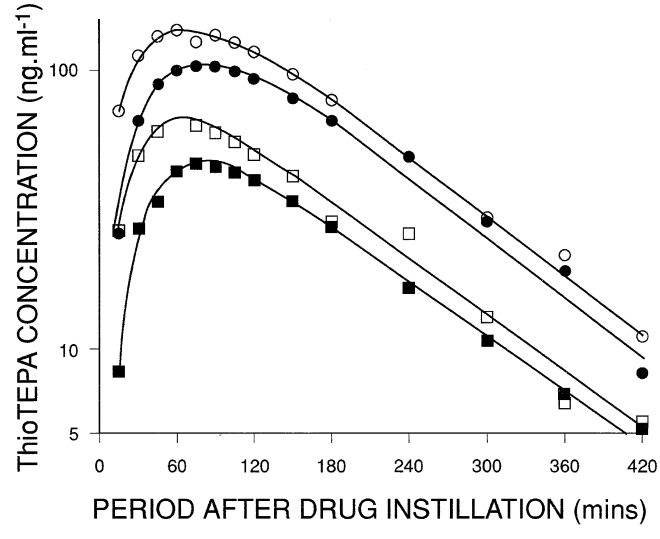

Fig. 1. Semilogarithmic fitted plots of mean plasma concentrations of ThioTEPA determined in 8 patients after administration of 30 mg/30 ml (□), 30 mg/60 ml (■), 60 mg/30 ml (○) and 60 mg/60 ml (●)

Table 2 Pharmacokinetic parameters for the absorption of ThioTEPA from the bladder. Data represent mean value ± SE ($n = 6$–8)

Dose/volume	K_a (min^{-1})	T_{max}(min)	C_{max}($\mu g\ ml^{-1}$)	$t_{1/2}$(min)
30 mg/30 ml	0.023 ± 0.004] *	73 ± 8] *	0.072 ± 0.009] *	81 ± 8
30 mg/60 ml	0.017 ± 0.001	91 ± 5	0.048 ± 0.008	82 ± 6
60 mg/30 ml	0.022 ± 0.004	72 ± 6	0.129 ± 0.025] *	89 ± 7
60 mg/60 ml	0.016 ± 0.003	92 ± 10	0.098 ± 0.015	91 ± 5
Analysis of variance (P):				
Dose	0.77	0.99	0.02	0.37
Volume	0.01	0.03	0.11	0.84

*$P < 0.05$ for between-treatment comparisons

and the drug was not detectable after 8 h. Negligible amounts of TEPA were found in plasma.

The pharmacokinetic parameters describing ThioTEPA uptake from the bladder and its circulation in the plasma are summarised in Table 2. Mean lag times were in the range of 4–10 min, which were not significantly different from zero and were therefore not considered. Mean peak plasma levels of ThioTEPA in plasma were observed at 72 and 73 min after installation of the 30-ml volume into the bladder and at 91 and 92 min after the 60-ml instillations. Differences in the time taken to reach mean peak plasma levels between the 30- and 60-ml instillations were significant at the lower 30-mg dose. Mean peak concentrations ranged from 48 to 130 $ng\ ml^{-1}$ and were influenced by both the dose and the volume such that C_{max} correlated with concentration. The half-life of the drug showed little variability with the dosing schedule and ranged from 81 to 91 min. There was no significant difference among the values recorded for clearance or for volume of distribution (Table 3).

In the bladder, between 6 and 32 mg of ThioTEPA was recovered at the end of the 2-h instillation (Table 4), indicating that approximately 50–80% of the drug had been degraded, adsorbed onto the bladder wall or absorbed into the circulation. Insignificant amounts of ThioTEPA were excreted over the next 6 h at the 30-mg dose, and between 1 and 2 mg was excreted after the 60-mg dose. More ThioTEPA was absorbed from the 60-mg dose (34.8 and 28.6 mg) than from the 30-mg dose (24.2 and 22.1 mg), indicating an association between dose and uptake, at least over this narrow range. At the higher dose (60 mg) the concentration of ThioTEPA, measured in the bladder at the end of the 2-h instillation was approximately 3 times higher than that determined at the lower dose (30 mg), indicating that a higher proportion of the drug had been adsorbed, absorbed or degraded at the lower dose (81% and 74% at the 30-mg doses as compared with 58% and 48% at the 60-mg doses). There was no significant change in the volumes obtained from the bladder at the end of treatment, which ranged between 130 and 150 ml. Low concentrations of TEPA were detected in the urine of five of the eight patients. Amounts of TEPA excreted in the urine, generally in the period of 4–6 h after the start of instillation, were variable and ranged from 1 to 145 μg.

Table 3 Volume of distribution and total body clearance of ThioTEPA. Data represent mean values ± SE ($n = 7$); total body clearance is expressed in ratio to bioavailability

Dose/volume	CL/F ($l\ min^{-1}$)	$V_{ss/F}(l)$
30 mg/30 ml	2.5 ± 0.27	239 ± 26
30 mg/60 ml	3.8 ± 0.68	281 ± 21
60 mg/30 ml	3.3 ± 0.81	278 ± 60
60 mg/60 ml	3.3 ± 0.61	329 ± 69
Analysis of variance (P):		
Dose	0.71	0.44
Volume	0.30	0.39

The AUC in the plasma and the bladder are shown in Table 4. In the bladder, as might be expected, the AUC to the tumour was approximately 4 times greater at the highest concentration of 2 $mg\ ml^{-1}$ (60 mg/30 ml) as compared with the lowest concentration of 0.5 $mg\ ml^{-1}$. The two concentrations of 1 $mg\ ml^{-1}$ (30 mg/30 ml and 60 mg/60 ml) might have been expected to produce similar AUCs in the bladder, but it was found that the higher dose/volume ratio (60 mg/60 ml) gave an AUC approximately 50% higher. These figures were obtained using a model based on first-order kinetics, assuming that the decrease in ThioTEPA concentration was due largely to the first-order processes of absorption and metabolism. The opposite extreme would be to assume that the major influence on the concentration in the bladder would be dilution by urine at a linear rate, in which case the AUC at both 1-$mg\ ml^{-1}$ doses would be similar: 63,044 and 70,256 $\mu g\ min\ ml^{-1}$ for the 30-mg/30-ml and 60-mg/60-ml doses, respectively. The AUC_∞ values in plasma were approximately 2-times greater at the higher (60-mg) doses and were significantly different (Table 4), indicating that the systemic exposure is approximately twice that resulting from the lower dose.

On the basis of the first-order model, the highest dose to the bladder and the highest therapeutic ratio (AUC bladder/AUC plasma) were achieved by giving 60 mg ThioTEPA in a 30-ml volume (Table 5). The AUC determined in the bladder (0–2 h) following

Table 4 ThioTEPA in the bladder at the end of the instillation and total TEPA excretion. Data represent mean value ± SE ($n = 5$–8)

Dose/volume	Amount of ThioTEPA in bladder at 2 h (mg)	Concentration of ThioTEPA in bladder at 2 h (μg ml^{-1})	Volume in bladder at 2 h (ml)	Amount of TEPA excreted over 8 h (μg)
30 mg/30 ml	5.8 ± 1.4 *	52.7 ± 14.2 *	130 ± 17	30.2 ± 23.5
30 mg/60 ml	7.9 ± 2.3 *	57.9 ± 11.9 *	153 ± 19	12.1 ± 10.6
60 mg/30 ml	25.2 ± 4.2	182.4 ± 30.6	149 ± 14	41.9 ± 22.9
60 mg/60 ml	31.4 ± 10.7	161.1 ± 41.7	148 ± 16	39.1 ± 18.7
Analysis of variance (P):				
Dose	0.02	0.01	0.73	0.07
Volume	0.38	0.57	0.51	0.52

*$P \leq 0.05$ for between-treatment comparisons

Table 5 Tumour exposure during instillation and total systemic exposure to ThioTEPA

Dose/volume	AUC (0–2 h) in bladder (μg min ml^{-1})	AUC_{∞} in plasma (μg min ml^{-1})	*AUC bladder (0–2 h)*/ AUC* plasma
30 mg/30 ml	37,005 ± 3,646 * *	12.8 ± 1.7 *	2,891.0
30 mg/60 ml	23,775 ± 2,314 *	9.4 ± 1.5 * *	2,529.3
60 mg/30 ml	90,087 ± 4,868 *	24.8 ± 5.1 *	3,632.5
60 mg/60 ml	53,652 ± 5,058	22.4 ± 4.0	2,395.2
Analysis of variance (P):			
Dose	< 0.001	0.01	
Volume	< 0.001	0.46	

*$P \leq 0.05$ for between-treatment comparisons

a dose of 60 mg in 60 ml was more than 2 times greater than that calculated following a dose of 30 mg in 60 ml and was also nearly 50% greater than that achieved giving 30 mg in 30 ml. However, little difference was found in the therapeutic ratio between these three doses (Table 5).

There was no clinical evidence of systemic toxicity as a result of intravesical chemotherapy. Myelosuppression was not observed, and in no case did the WBC, platelet count or haemoglobin value fall below the normal level.

Discussion

This study describes the influence of drug dose and volume on systemic uptake and tumour exposure following instillation of ThioTEPA into the bladder of patients with recurrent superficial transitional-cell cancer. The pharmacokinetics of ThioTEPA following intravesical instillation are complicated because the drug enters the systemic circulation through the bladder and can be recycled back into the bladder. Recoveries at the end of the 2-h instillation were highly variable, ranging from 20% to 50%, confirming our previous study in which we found that between 20% and 78% of the drug was recoverable [13].

Our earlier study was the first to measure the pharmacokinetics of ThioTEPA during intravesical chemotherapy using a direct assay for the drug, and that study used a dose of 30 mg in 30 ml of water. The results obtained in the present study with the same dose are not identical to the earlier data but are similar. A comparison of the previous data with the present data, respectively, reveals the following: T_{max}, 58 and 73 min; C_{max}, 101 and 72 ng ml^{-1}; $t_{1/2}$, 110 and 81 min; volume of distribution, 209 and 239 l; and total body clearance, 1.7 and 2.5 l min^{-1}. The differences are probably accounted for by the different patient populations, the small groups and the relatively large variations between individuals. Pharmacokinetic data have also been obtained following systemic administration of ThioTEPA in human [5, 9, 14, 16] and animals [1, 7, 15]. Our data fit with the results of these earlier studies, although it would not be valid to make a direct comparison because of the recycling of the drug from the bladder following intravesical administration.

TEPA is the primary metabolite of ThioTEPA and is thought to contribute significantly to the toxicity and antitumour activity of the latter following systemic administration. In both the present and our previous study, only minute quantities of TEPA were detected. ThioTEPA decomposes in urine in a pH- and temperature-dependent manner, probably by successive conversion of the aziridine rings to alkylating compounds

containing 2-chloroethyl species [4]. After 2 h of incubation at 37°C in urine in vitro, ThioTEPA concentrations fell by 10% at pH 6 or 7 and by 40% at pH 5 [4]. The alkylating activity was stable over this period. The metabolism of ThioTEPA in urine may differ from that in the systemic circulation, and the roles of ThioTEPA metabolism, pH and absorption in relation to response in individual patients is not known. The local and systemic actions of the drug may be mediated in part by other alkylating metabolites.

Since similar plasma AUC values were obtained for the two 30-mg doses and these were approximately half of those achieved at the 60-mg doses, systemic exposure to the drug appears to be controlled by the amount of drug instilled into the bladder. However, as previously found, there was great variation in the proportion absorbed between patients, perhaps due to differences in the state of the bladder wall. As uptake cannot be predicted in individual patients, in assessments of potential risk the assumption must be made that all of the drug could pass into the systemic circulation.

There was no evidence of local or systemic toxicity to any patient at any dose rate. Therefore, at these doses, exposure to the tumour is the most important parameter to consider, and theoretically the aim should be to produce the highest AUC possible in the bladder. As might be expected, the highest concentration, $2\,mg\,ml^{-1}$, gave an AUC in the bladder that was approximately 4 times greater than that achieved at the lowest concentration, $0.5\,mg\,ml^{-1}$. However, on the basis of the pharmacokinetic model used (first-order), dose/volume also had a significant impact on the AUC to the tumour, in that 60 mg/60 ml produced an AUC value 50% higher than that obtained at 30 mg/30 ml.

ThioTEPA is absorbed from and can recirculate into the bladder, both of which are first-order processes. The greater the extent of absorption of ThioTEPA, the more closely will the pharmacokinetic approximate to this model, as the influence of urinary dilution will be less marked. However, the evidence for recirculation is limited. Urinary clearance of ThioTEPA following systemic administration accounted for only 1.5% of the delivered dose in breast cancer patients [5] and for 0.14% of the dose in a study of ovarian cancer patients [10]. However, in the breast cancer study, 4.2% of the delivered dose was recovered in urine as TEPA and the alkylating activity accounted for a further 23.5%. These data indicate that nearly 30% of the delivered dose could be found in urine, but predominantly as unidentified metabolites [5]. If it is assumed that there is no recirculation into the bladder, tumour exposure can be predicted from AUC values calculated according to simple first-order decay. If, however, urinary dilution is the major influence, tumour exposure will be greater. Nevertheless, the conclusions from this study would not alter, although the differences between the 30-mg/30-ml dose and the 60-mg/60-ml dose in terms of the AUC in the bladder would be much less marked. The first-order model would not be valid for drugs such as Adriamycin and mitomycin C, although the same conclusions would again apply. Drug concentration would remain the dominant factor influencing the AUC to the tumour. For Adriamycin and mitomycin C the major factor influencing the drug concentration in the bladder is dilution by urine [3, 6, 19]. Consequently, for these drugs there would be little difference in th AUC between 60 mg/60 ml and 30 mg/30 ml.

In conclusion, it is possible to increase the exposure of bladder cancer to ThioTEPA by 55–68% without significantly increasing systemic toxicity. This was achieved by instilling the same amount of drug in half the volume. These benefits will be greater for drugs such as Adriamycin and mitomycin C because they are not absorbed into the circulation.

In the clinic the dose of intravesical chemotherapy is often described as the weight of drug instilled into the bladder. Consequently, variables such as drug concentration and exposure period are not recorded. Consideration of these variables in the design of schedules for intravesical chemotherapy might result in greater benefit to the patient at a lower cost.

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