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Absorption of Epi-Doxorubicin After Intravesical Administration in Patients with *in situ* Transitional Cell Carcinoma of the Bladder

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Abstract—*Nine patients with* in situ *bladder cancer (TIS) were treated by intravesical instillation of epi-doxorubicin (epi-DOX). The amount of anthracycline in* 1 ml *plasma was in the nanogram range.* 78.9 ± 12.0% *and* 84.2 ± 10.6% *of the administered dose* (30 *and* 50 mg, *respectively) could be recovered.*

INTRODUCTION

INTRAVESICAL instillation of doxorubicin (DOX) has been widely used, either with a therapeutic intent or to prevent recurrent bladder cancer. The therapeutic approach of intravesically administered DOX has mainly been used for *in situ* bladder cancer (TIS) [1, 2]. Prevention of recurrent bladder cancer has been applied in stage Ta and T1 following transurethral resection (TUR) of the tumor [3, 4, 5]. In the absence of further therapy following TUR for superficial Ta–T1 tumors the recurrence rate is 40–70%, most of these tumors will occur within 12 months [6]. The aim of additional local chemotherapy is to prevent recurrences.

Theoretically, a possible disadvantage of intravesical chemotherapy may be the occurrence of systemic side-effects due to drug uptake through the bladder wall and its capillary plexus into the blood. It has been shown with radiolabeled DOX that the concentration gradient between the bladder lumen and the plasma compartment is approx. 40.000 [7]. The low absorption of DOX through the bladder wall explains the lack of undesired systemic side-effects of DOX such as myelosuppression [7, 8]. However, the limiting factor for intensive intravesical chemotherapy appears to be a chemical cystitis. The occurrence of chemical cystitis is 4% in patients treated by weekly instillations, but increases to 26% if given 24 hr after TUR and twice during the first week after surgery [9, 10].

In the present study, patients with TIS bladder cancer were treated with intravesical epi-DOX. This doxorubicin analog has an antitumor spectrum similar to that of DOX, but appears to exert less toxicity to the heart and other organs than the parent drug [11]. In the event of this analog also proving to cause less local cystitis, but with maintenance of a high concentration gradient between the bladder lumen and plasma similar to DOX, the possibility might be created for early and repeated instillation regimens. The purpose of the present study was to examine the recovery of the drug from the bladder and its penetration into the plasma compartment after intravesical administration.

MATERIALS AND METHODS

Nine patients with TIS bladder cancer were treated with 30 mg and/or 50 mg epi-DOX, given intravesically. Local treatment was repeated weekly for 8 weeks. All patients entering the study had a positive urine cytology (Papanicolau IV–V) and an *in situ* carcinoma diagnosed by a biopsy of the bladder wall. The bladder capacity of all patients was greater than 250 ml with no residual urine. Patients with proven urinary tract infections were treated with appropriate antibiotics prior to the intravesical chemotherapy.

Epi-DOX (30 or 50 mg) was dissolved in 50 ml sterile saline using a closed system. The solution was drawn in a 50 ml syringe and the volume of the solution administered to the patient was registered. An aliquot of the solution was kept for drug analysis (U1). The catheter was clamped

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for 60 min. Thereafter the content of bladder was collected (U2) and the bladder was irrigated with 100 ml sterile saline (U3). The first urine voided after removal of the system was also collected (U4). All fluids of 17 courses with 30 mg of epi-DOX and 15 courses with 50 mg of epi-DOX were collected. The volumes of the fluids were measured and aliquots (1–4 ml) were collected in polypropylene tubes and stored at −20° C until the assays were carried out. During 8 courses with 30 mg of epi-DOX and 5 courses with 50 mg of epi-DOX additional blood samples were collected in heparinized tubes in 0, 30, 45, 60, 75, 90, 120, 150, 210 and 270 min. These samples were immediately centrifuged at 4000 ***g*** for 10 min. The plasma was transferred to polypropylene tubes. Plasma samples were stored at −20° C until the assays were carried out.

Epi-doxorubicin levels in plasma samples were measured with an improved assay procedure [12] based upon a previously described anthracycline assay [13]. After thawing, the urine samples were sonificated for 10 min and immediately centrifuged at 4000 ***g***, 4° C during 10 min. The supernatant was acidified with phosphoric acid to pH 2.5 and 30 µl of the supernatant was directly injected onto an analytical HPLC column. Plasma samples were extracted prior to injection onto the column with C-18 SEP-PAK cartridges (Waters Millipore, Etten-Leur, The Netherlands) pretreated with 5 ml methanol, 5 ml aqua dest. and 5 ml 0.018 M NaH_2PO_4 pH 4.0/acetonitrile (9/1, v/v). One ml of plasma was injected and washed with 2 ml of the buffer/acetonitrile mixture. The retained epi-DOX was eluted with 5 ml of a methanol/chloroform (3 1, v/v) mixture. This solution was evaporated to dryness under a stream of air at 50° C. The residue was redissolved in 50 µl 0.018 NaH_2PO_4 pH 4.0/acetonitrile mixture (9/1, v/v) of which 30 µl was injected onto the column. All samples were measured in duplicate. The HPLC system consisted of a 6000A pump, a WISP 710B injection system, a Data Module and a System Controller (Waters Millipore, Etten-Leur, The Netherlands) provided with a 4.6 × 100 mm RP C-18 3 µm column (Chrompack, Middelburg, The Netherlands) connected to a precolumn 4.0 × 4.0 mm LiChrosorp RP-18 5 µm (Merck, Amsterdam, The Netherlands) and a fluorescence detector F-1000 (ex. 480 nm em. 580 nm) from Merck/Hitachi, The Netherlands. An isocratic eluent was used, consisting of 0.018 M NaH_2PO_4 pH 40/acetonitrile (2.5/1, v/v) at a flow rate of 1 ml/min. Plasma samples spiked with epi-DOX (5×10^{-8}–10^{-6}M) were used for calibration curves. The detection limit for epi-DOX was 0.5 ng/ml plasma. Due to differences in extraction procedure and detection, early samples were analyzed with a slightly higher detection limit (up to 2 ng/ml) (see Table 3).

Table 1. *Amounts of epi-DOX (mean ± S.D. and range), recovered and absorbed after* 30 *and* 50 *mg dosages*

Dose of epi-DOX	Amount of drug recovered* (mg)	Amount of drug absorbed† (mg)	Overall recovery‡ (%)
30 mg (n=17)	21.5 ± 4.2 15.3 – 31.6	5.2 ± 3.4 0.0 – 11.1	78.9 ± 12.0
50 mg (n=15)	38.3 ± 5.6 24.4 – 45.7	7.4 ± 4.9 2.1 – 15.8	84.2 ± 10.0

*U2+U3+U4, †U1−(U2+U3+U4), ‡ $\frac{(U2+U3+U4)}{U1} \times 100$.

RESULTS

The mean instilled dose of epi-DOX, calculated from the U1 samples, of patients who were scheduled to receive 30 mg was 26.7 ± 2.3 mg (range: 21.4–31.2 mg), while the mean recovery calculated from U2, U3 and U4 samples was 78.9 ± 12% (range: 60–101%). The mean dose of epi-DOX instilled in patients who should have received 50 mg epi-DOX was 45.8 ± 5.8 mg (range: 37.0–56.6 mg), while the mean recovery calculated from U2, U3 and U4 samples was 84.2 ± 10.0% (range: 63.4–99%). A complete survey of these data including means, ranges and recoveries is given in Tables 1 and 2.

Penetration of epi-DOX into the blood during and following intravesical instillations (from 0 to 270 min) was low. The results of 13 treatment courses (= 9 patients) listed in Table 3, show a large inter-individual and intra-individual variation. The plasma concentrations were close to the detection limit of the assay, except for those of patient No. 6, in whom higher levels were observed. The limited number of patients and the low plasma concentrations of epi-DOX did not allow correlation of dose and plasma concentrations, although there seems to be some evidence that in patients with recoveries approaching 100% no epi-DOX is detectable in plasma (data not shown). Degradation products or metabolites of epi-DOX could neither be detected in urine nor in plasma.

DISCUSSION

This study shows a negligible absorption of intravesical epi-DOX through the transitional cell epithelium into the capillary plexus of the bladder. These findings are in agreement with those reported for DOX [14–16]. Lesions in the tissue barrier between the urine and the capillary plexus, which may be caused by infection, chemical cystitis, radiotherapy, transurethral procedures and malignancy itself have been associated with an increased plasma concentration of anti-cancer drugs after intravesical

Table 2. Epi-DOX concentrations (mean ± S.D. and range) in samples following 30 and 50 mg dosages

Dose of epi-DOX	Instillation fluid U1 (mg)	Recovered instillation fluid U2 (mg)	Irrigation fluid U3 (mg)	Initial urine voided U4 (mg)
30 mg (n=17)	26.7 ± 2.3 21.4 – 31.2	20.8 ± 4.9 8.5 – 31.0	1.4 ± 0.2 0.1 – 6.75	0.03 ± 0.04 0.002 – 0.14
50 mg (n=15)	45.8 ± 5.8 37.0 – 56.6	36.6 ± 5.8 22.8 – 45.4	1.4 ± 0.8 0.31 – 3.54	0.29 ± 0.37 0.004 – 1.97

Table 3. Plasma concentrations of epi-DOX in ng/ml *at* 0–270 min *following intravesical administration of* 30 *and* 50 mg *of the drug*

Patient No.	Dose (mg)	0	30	45	60	75	90	120	150	210	270
1	30	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0
2	30	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0
3	30	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< .20	< 2.0	< 2.0	< 2.0	< 2.0
4	30	< 1.0	1.2	< 1.0	< 1.0	1.8	1.1	1.8	1.2	*	*
5	30	< 1.0	< 1.0	< 1.0	< 1.0	1.9	1.4	< 1.0	< 1.1	*	*
6	30	< 1.0	< 1.0	< 1.0	0.9	6.0	3.6	4.4	3.1	4.1	7.2
7	30	< 0.5	11	24	34	16	262	< 0.5	< .05	< 0.5	60
8	30	< 0.5	4.8	1.0	1.0	1.6	< 0.5	2.9	< 0.5	1.6	< 0.5
9	50	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0	< 2.0
10	50	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	*	*
11	50	< 0.5	3.2	3.2	< 0.5	< 0.5	< .05	< 0.5	< 0.5	< 0.5	3.2
12	50	< 0.5	1.5	2.9	3.7	2.2	2.2	1.5	2.2	1.8	1.8
13	50	< 0.5	5.7	< 0.5	< 0.5	< 0.5	5.7	< 0.5	2.9	< 0.5	5.7

*Not sampled.

administration [17–19]. In the present study we detected in 6 out of 13 courses more than 2 ng epi-DOX/ml plasma. The plasma uptake of epi-DOX after intravesical administration was in the ng-range and did not exceed 10 ng/ml in 12 out of 13 treatment courses. Uptake of epi-DOX into the circulation was not related to urinary tract infections or chemical cystitis as in such patients instillation was delayed.

Finally, none of these patients had a stage beyond TIS. A possible explanation, but not necessarily the only one for the detection of traces of the drug in the plasma, might be the fact that the urothelium had been affected by repeated transurethral procedures. We found in one patient (No. 7; Table 3) relatively high amounts of epi-doxorubicin without any good explanation for this kinetic. The abnormally high levels of epi-DOX measured in plasma samples of this patient are probably caused by a technical error due to contamination.

Of interest, patients who were to receive 30 mg epi-DOX actually received a mean dose of 26.7 mg with a range of 21.2–31.2 mg. Patients who should have received 50 mg epi-DOX received a mean dose of only 45.8 mg with a range of 37.0–56.6 mg. In other words, the administered epi-DOX dose varied beween 71 and 113% of the scheduled dose. Most probably the main reasons for lower actual doses than planned were residues remaining in the original vials and in the bag from which the saline solution was transferred into the syringe. The recoveries in both groups of patients receiving the 2 different doses of epi-DOX, were similar. The main contribution to the total recovery of the drug was present in the U2 samples (more than 95%) while only small amounts of the drug were removed from the bladder by irrigation (U3) or the first urine voided (U4). Possibly, epi-DOX is moving from the superficial mucosal layer to the capillary plexus, and/or is, perhaps, purged by the normal urine.

If one takes into account that the maximum acceptable cumulative dose of epi-DOX is 800 mg/m^2 (for DOX 550 mg/m^2 [20]) for pulse i.v.doses of the drug in order to prevent cardiotoxicity [21], it is obvious that no risk is involved with the doses applied in the present study. Considering the fact that peak levels observed with pulse doses are most likely responsible for cardiotoxicity, the absence of initial peaks may be an additional reassurance. Moreover, the data form a firm basis to evaluate escalating doses of the drug in instillation studies on the pharmacological aspects, antitumor activity

and possible development of chemical cystitis. In case chemical cystitis appears to occur less frequently than with DOX, a higher dose and repeated instillations may well result in improved antitumor effect of epi-DOX.

Data on the therapeutic efficacy of epi-DOX will not be published until a 1-year follow-up of the patients, treated intravesically for *in situ* carcinoma of the bladder, is achieved.

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