Intravesical administration of pirarubicin against superficial bladder cancer: Relationship between tumor tissue concentration and exposure time in the bladder or therapeutic effect

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Abstract. The aim of this study was to investigate the relationship between tissue concentrations and exposure times or therapeutic effect of an anthracycline anticancer drug, pirarubicin, in bladder cancer tissue after single intravesical administration against superficial bladder cancer. The concentrations of pirarubicin in tumor tissues and serum were measured at designated collection times after a single intravesical administration of pirarubicin (30 mg) in 22 patients with superficial bladder cancer. A wide range of concentrations of pirarubicin in bladder cancer tissue was observed (2.3-125 μg/g of tissue), although serum pirarubicin concentrations were not detected in any of the patients. Recurrence of superficial bladder cancer after transurethral resection of the bladder tumor (TUR-BT) was observed in 2 patients (9%). The concentration of pirarubicin in the tumor tissue tended to be higher as the exposure time increased. There was a weak relationship between the pirarubicin tissue concentration and tumor size. However, no significant relationship between tissue pirarubicin concentrations and the prophylactic effect against intravesical recurrence of bladder cancer after TUR-BT was observed. All patients had no adverse events, such as bladder irritation and local toxicity, caused by the treatment with pirarubicin. These findings suggest that prior to single intravesical administration of pirarubicin to patients with superficial bladder cancer the exposure time and tumor size should be considered.

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Introduction

Superficial bladder cancer is mainly treated by transurethral resection of the bladder tumor (TUR-BT). Yet, the intravesical recurrence rate of bladder cancer after TUR-BT is reported to be 50-70% (1-3). Therefore, prophylaxis of the high frequency of recurrence after TUR-BT is important. Bacillus Calmette-Guérin (BCG), doxorubicin, mitomycin, epirubicin, thiotepa and pirarubicin are currently used as intravesical instillation chemotherapy agents to reduce the recurrence rate of bladder cancer (4-13).

Pirarubicin (Fig. 1), an anthracycline drug which has been shown to have a high affinity to bladder tumor cells or tissues (14-16), is a drug used widely for intravesical instillation chemotherapy. The concentration of pirarubicin in the tumor tissue becomes markedly high in a relatively short time after administration into the bladder (15). It is reported that the intravesical instillation with pirarubicin produces an antitumor effect against bladder cancer, and that the prophylactic effect of recurrence in the bladder after intravesical instillation chemotherapy with pirarubicin is higher than that after TUR-BT only (17). Moreover, short-time intravesical instillation chemotherapy is performed to obtain a greater antitumor effect and to prevent an increase in side effects, as this method has the advantage of a higher penetration of pirarubicin into bladder tumor tissues (18).

Conditions for optimal injection of pirarubicin, a concentration-dependent anticancer drug, has yet to be established. The role involving the exposure time of the bladder to pirarubicin in the prophylactic effect against recurrence of bladder cancer has been extensively studied (17-19), whereas the contribution of the concentration of pirarubicin in bladder tumor tissue to the prophylactic effect against recurrence of bladder cancer remains unclear.

As part of a program for the development of guidelines for the safe use of pirarubicin and effective intravesical chemotherapy with pirarubicin for the prevention of recurrence of bladder cancer, the present study investigated the relationship between the concentration of pirarubicin in bladder tumor tissues and its exposure time in the bladder or therapeutic effect after intravesical instillation chemotherapy with pirarubicin.

Materials and methods

Patients. Characteristics of the patients enrolled in this study are summarized in Table I. Twenty-two patients with superficial bladder cancer, who were hospitalized in the Department of Urology of Aichi Medical University Hospital, Tokoname Municipal Hospital and Sakashita Hospital, between August 2008 and March 2010, participated in this study. The patients included 21 males and 1 female, aged 51-92 years (mean 70). All patients were diagnosed with transitional cell carcinoma with papillary and initial and primary tumors with superficial (Ta, T1) tumors and a non-invasive tumor. From histopathological findings, the tumor grade was found to be G1 in 5 patients and G2 in 17 patients. The tumor stage was shown to be pT1 in 2 patients and pTa in 20 patients. Solitary and multiple tumors were found in 17 and 5 patients, respectively. The study was approved by the Institutional Review Board of Aichi Medical University School of Medicine, and written informed consent was obtained for all participants prior to enrollment in the study.

Drugs. Pirarubicin injection (therarubicin, 10 mg/20 mg of pirarubicin per injection; Meiji Seika, Tokyo, Japan) and pirarubicin hydrochloride (Wako Chemicals, Tokyo, Japan) were used in this study. Pirarubicin (30 mg) was dissolved in 30 ml of distilled water for the injection (1 mg/ml). All other reagents were commercially available, of analytical grade, and were used without further purification.

Drug administration. Pirarubicin (30 mg) was administered transurethrally for 8 min in 1 patient, 9 min in 1 patient, 10 min in 1 patient, 15 min in 3 patients, 20 min in 2 patients, 25 min in 1 patient, 30 min in 10 patients and 35 min in 1 patient (Table II). Bladder cancer tissue and blood samples were collected at the designated intervals described above. The removed bladder cancer tissues were washed immediately with ice-cold saline five times. The washed tissue and serum samples obtained from the patients were stored at -80˚C until analysis.

Drug analysis. The pirarubicin concentrations in the plasma and tissue were determined by high-performance liquid chromatography (HPLC). The apparatus used for HPLC was a Shimadzu LC-10A system (Kyoto, Japan) equipped with a fluorescence detector (RF-10AXL; Shimadzu) (excitation, 273 nm; emission, 464 nm) consisting of an LC-10A liquid pump and an SIL-10A autoinjector. The conditions were as follows: column, a Cosmocil 5C18 column (4.6 by 150 mm; Nacalai Tesque, Kyoto, Japan); mobile phase, 20 mM potassium dihydrogen phosphate-acetonitrile [1:1 (vol/vol)] solution; column temperature (CTO-10AC; Shimadzu), 50˚C; flow rate, 1.0 ml/min.

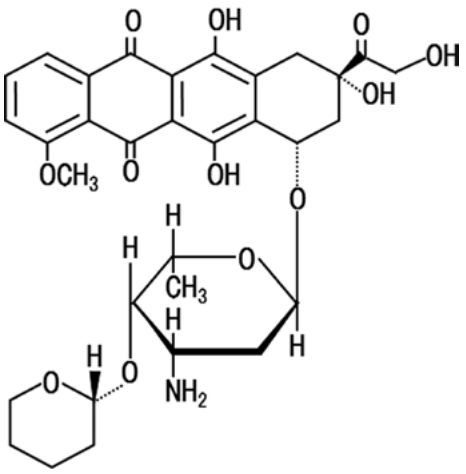

Figure 1. Structure of pirarubicin.

Table I. Characteristics of the patients enrolled in this study.

Patients	
Males	21
Females	1
Age, in years	
Range	51-92
Mean	70.0
Tumors	
Solitary	17
Multiple (2)	5
Grade	
G1	5
G2	17
Stage	
pTa	20
pT1	2

Each bladder tissue sample (0.002-0.215 g) was weighed and homogenized with ice-cold saline using a tight homogenizer (20 strokes up and down) and adjusted to 0.5, 1 or 2 ml by saline according to each tumor weight. Each sample (100 μl) of serum and diluted tissue homogenates, 50 μl of 0.1 M $NaHCO_3$-saturated NaCl solution and 150 μl of acetonitrile were mixed vigorously and centrifuged at 21.880 x g for 10 min at 4˚C. After centrifugation, the supernatant (50 μl) was injected into the column.

The standard curves for this assay were shown to be linear for the concentrations measured (500, 1,000, 2,000 and 4,000 ng/ml) with a correlation coefficient of 0.999. The within-day and between-day coefficients of variation (CV) for this assay were <8%. No interference with the peak of pirarubicin was observed in any samples. The quantitative limit of this assay was 500 ng/ml.

Clinical evaluation. Adverse events, such as frequent urination, pain on urination, hematuria and a feeling of residual urine were observed. The frequency and severity of these

Table II. Characteristics of the patients that received single intravesical therapy with pirarubicin.

Patient	Gender	Age (years)	Grade	Stage	No. of tumors	Size (mm)	Retention time (min)	Concentration (μg/g tissue)	Recurrence	Follow-up (months)
1	M	66	G2	pTa	1	50	8	10.6	+	3
2	M	85	G2	pT1	1	24	9	44.4	-	16
3	M	67	G1	pTa	2	20	9	5.6	-	17
4	M	75	G2	pTa	1	39	10	7.7	x	x
5	M	74	G1	pTa	1	15	12	16.9	-	13
6	M	70	G2	pTa	2	19	15	65.6	x	x
7	M	72	G2	pTa	1	15	15	20.4	-	14
8	M	54	G2	pTa	1	5	15	13.9	-	20
9	M	53	G2	pTa	2	24	20	4.9	-	14
10	F	58	G2	pTa	1	20	20	17.9	x	x
11	M	67	G2	pTa	1	17	25	23.0	-	13
12	M	92	G2	pTa	1	15	30	13.8	-	29
13	M	51	G2	pTa	1	30	30	2.3	-	29
14	M	71	G2	pT1	2	34	30	47.7	+	5
15	M	80	G2	pTa	1	15	30	4.7	-	29
16	M	87	G2	pTa	1	23	30	125.0	x	x
17	M	73	G1	pTa	1	10	30	79.6	-	26
18	M	61	G1	pTa	1	15	30	11.9	-	14
19	M	65	G2	pTa	1	13	30	26.7	-	26
20	M	81	G2	pTa	2	8	30	16.3	x	x
21	M	71	G1	pTa	1	5	30	125.0	-	20
22	M	67	G2	pTa	1	6	35	45.9	-	10

Recurrence was observed for 1 year after TUR-BT. x, not followed up.

events were also investigated. The therapeutic effect was evaluated between the date of surgery and January 2011 for the non-recurrence period. Cystoscopy and urinary cytology in all patients were performed at 3-month intervals during the first year.

Statistical analysis. Data are represented as observed values. Statistical analysis was performed using Stat View (Abacus Concept, Barkeley, CA, USA).

Results

Various clinical data for each patient receiving a single intravesical therapy with pirarubicin are summarized in Table II. Concentrations of pirarubicin in bladder cancer tissues showed a wide range from 2.3 to 125 μg/g tissue, whereas no serum concentrations of pirarubicin were observed in any of the patients.

Weak relationships, not statistically significant, were observed between pirarubicin tissue concentrations and exposure time of pirarubicin in the bladder or tumor size. As shown in Fig. 2A, the concentration of pirarubicin in tumor tissues tended to be higher as the exposure time was prolonged, whereas the concentration tended to be lower as the tumor size increased (Fig. 2B). However, there was no relationship between pirarubicin tissue concentrations and prophylactic effect against recurrence of bladder cancer after TUR-BT.

No severe adverse events caused by treatment with pirarubicin were noted in all patients.

Discussion

There is no detailed information regarding the effect of the exposure time of pirarubicin in the bladder on tissue concentrations in patients with superficial bladder cancer. In the present study, to evaluate this issue, the concentrations of pirarubicin were measured in the tumor tissues and serum at different collection times after a single intravesical administration of pirarubicin in 22 patients with superficial bladder cancer.

In the present study, a weak relationship between pirarubicin tissue concentrations and exposure time of pirarubicin in the bladder or tumor size was observed. Unexpectedly, the concentrations of pirarubicin in the tumor tissues had a tendency to be lower as the tumor size increased, indicating that the uptake of pirarubicin into tumor tissue decreases as the tumor size increases. We postulated the possibility that concentrations of pirarubicin in the bladder did not reach a steady state. The present study found that in the 2 patients who presented with a recurrence of bladder cancer the tumor size was larger than 30 mm. These results corroborate those of Kanayama *et al* (20), who reported that a higher incidence of the recurrence of bladder cancer was observed in patients having 5 or more tumors, or in patients with a tumor size larger than 30 mm. Therefore, tumor size should be considered when

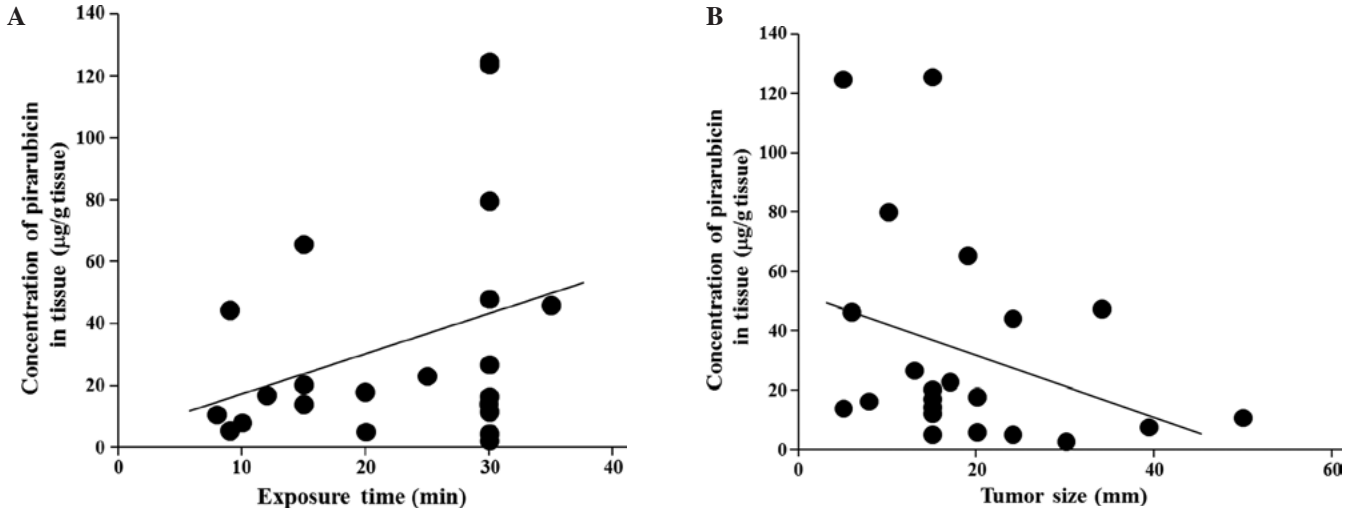

Figure 2. (A) Relationship between tissue concentrations of pirarubicin and exposure time in the bladder. The regression line was calculated as y = 1.25x + 5.15. The correlation coefficient (r) was 0.316. (B). Relationship between tissue concentrations of pirarubicin and tumor size. The regression line was calculated as y = -1.05x + 52.9. The correlation coefficient (r) was 0.327.

intravesical chemotherapy with pirarubicin against superficial bladder cancer is conducted. Assuming that pirarubicin is distributed into tumor tissues by a passive diffusion system, we should consider the presence of an efflux pump in cases of tissues of small tumors exhibiting lower concentrations of pirarubicin. The susceptibility of pirarubicin to tumor tissues of each patient should also be considered.

There are several reports regarding the involvement of certain efflux drug transporters in bladder cancer (21-25). It is well known that one of the drug transporters, ABCB1/P-glycoprotein, an ATP-binding cassette transporter protein, acts as an efflux pump for various drugs, such as *Vinca* alkaloid and anthracycline anticancer drugs. This transporter is expressed in anticancer drug-resistant tumor cells. In the present study, relatively low concentrations of pirarubicin in bladder tumor tissues were observed in several patients who were administered well-known P-glycoprotein substrates, such as atorvastatin, fexofenadine, diltiazem and itraconazole. Considering that pirarubicin is a typical P-glycoprotein substrate, the observed low concentrations of pirarubicin in bladder tissues may be explained by efflux of pirarubicin from tumor tissues overexpressing P-glycoprotein or by efflux of pirarubicin by competitive inhibition of P-glycoprotein substrates. These observations suggest the need to measure the expression levels of P-glycoprotein in the tumor tissues of each patient before initiating intravesical chemotherapy with pirarubicin.

The present findings are, at least in part, supported by the results of Maruyama *et al* (26), who reported that the growth of T-24 cells *in vitro* was inhibited by the presence of pirarubicin in a dose- and time-dependent manner. The present results corroborate those of Yamamoto *et al* (27), who reported that pirarubicin was not detected in the plasma after single intravesical administration of pirarubicin, and by a report by Okamura *et al* (17), who reported that there were no severe local toxicities after a single dose of pirarubicin for 60 min.

In conclusion, the results obtained from the present study suggest that the concentration of pirarubicin in bladder cancer tissue appears to be dependent on the exposure time of pirarubicin in the bladder after single intravesical administration. Considering that intravesical administration of anticancer drugs is useful for the therapy of superficial bladder cancer with considerably high recurrence, further studies are required to investigate the optimal dosage regimen for intravesical administration of pirarubicin using a large number of patients with superficial bladder cancer.

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