Single Short Retention Instillation of Pirarubicin Prevents Intravesical Recurrence of Low-risk Non Muscle Invasive Bladder Cancer

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Abstract. *Background:* This study evaluated the efficacy of a single instillation of pirarubicin with a short retention time for preventing intravesical recurrence of low-risk non-muscle-invasive bladder cancer. *Patients and Methods:* We analyzed 165 patients with low-risk non-muscle-invasive bladder cancer who underwent transurethral surgery. Single instillation of pirarubicin with 15-min retention time immediate after surgery was performed in 47 (28%) patients. The other patients (118, 72%) were treated without instillation therapy. The primary endpoint was recurrence-free survival. *Results:* Median overall follow-up was 50 (range=6-134) months. Recurrence-free survival at 1 and 5 years was 91% and 72%, and 79% and 54% in the group treated with pirarubicin, and that treated with surgery alone, respectively ($p=0.031$). Cox's hazard analysis revealed lack of instillation and larger tumor size (>10 mm) as significant factors for risk of recurrence. No adverse events regarding

intravesical chemotherapy were observed. *Conclusion:* Pirarubicin instillation with 15-min retention time can prevent intravesical recurrence of low-risk bladder tumors.

Bladder cancer is the 10th most common cancer worldwide, with approximately 550,000 new cases diagnosed in 2018 (1). About 75% of patients have non-muscle-invasive bladder cancer (NMIBC) at initial diagnosis and usually undergo transurethral resection of bladder tumor (TURBT) (2). However, a high intravesical recurrence rate is the major clinical problem, and adjuvant intravesical chemotherapy and immunotherapy are required in order to reduce recurrence and progression. Low-risk NMIBC is defined in clinical guidelines by the presence of small (<3 cm), solitary, low-grade non-invasive papillary carcinoma (Ta tumor); the disease is associated with lower intravesical recurrence and progression rates when compared to intermediate- and high-risk NMIBC (3-5). Immediate postoperative single instillation (IPSI) of chemotherapeutic agents is strongly recommended to prevent intravesical recurrence of low-risk NMIBC (3-6). In spite of its established efficacy in reducing intravesical recurrence, IPSI is infrequently used in clinical practice (7).

Pirarubicin (4'-O-tetrahydropyranyladriamycin), a semi-synthetic anthracycline glycoside, is a chemotherapeutic agent that is used for intravesical therapy (8). Its prophylactic effect is similar to that of mitomycin C (MMC) and epirubicin (9). Pirarubicin is taken up rapidly in both cultured tumor cells and bladder tumors in situ (10, 11). In

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Table I. Characteristics of patients treated with transurethral resection of bladder tumor (TURBT) only or with TURBT plus pirarubicin.

	Overall	Pirarubicin	TURBT alone	
Case number	165	47	118	p-Value
Age, years				
Median (range)	68 (40-95)	68 (47-90)	68 (40-95)	0.884
Gender, n (%)				
Male	134 (81.2)	41 (87.2)	93 (78.8)	0.272
Female	31 (18.7)	6 (12.8)	25 (21.2)	
Tumor size, mm				
Median (range)	10 (1-25)	10 (2-25)	10 (1-25)	0.854
Voided urine cytology, n (%)*				
Positive	16 (14.7)	7 (16.7)	9 (13.5)	0.782
Negative	93 (85.3)	35 (83.3)	58 (86.5)	
Grade (WHO 1973), n (%)**				
1	91 (55.8)	23 (50.0)	68 (58.1)	0.384
2	72 (44.2)	23 (50.0)	49 (41.9)	
Follow-up for censored patients, months				
Median (range)	50 (6-134)	51 (9-113)	50 (6-134)	0.676

Data not available: *56 and **2 patients.

Table II. Results of univariate and multivariate analyses for intravesical recurrence of non-muscle-invasive bladder cancer.

		Univariate		Multivariate	
		p-Value	HR	95% CI	p-Value
Age	≤68 vs. >68 Years	0.961			
Gender	Male vs. female	0.914			
Cytology	Negative vs. positive	0.398			
Grade	1 vs. 2	0.650			
Tumor size	≤10 vs. >10 mm	0.002	2.33	1.40-3.87	0.001
Instillation	Pirarubicin vs. none	0.036	2.07	1.10-3.89	0.024

Statistically significant p-values are shown in bold.

this study, we compared the efficacy of a single pirarubicin instillation with a short retention time to TURBT alone in patients with low-risk NMIBC.

Patients and Methods

Patient population. One hundred and sixty-five patients with low-risk NMIBC who were treated at Shiga University of Medical Science Hospital and affiliated hospitals between 2006 and 2015 were included in this study. The histological diagnosis of all patients was urothelial carcinoma. Low risk was defined according to the Japanese Urological Association clinical practice guidelines for bladder cancer (3), which includes a single lesion, primary lesion, size <3 cm, Ta, low grade (12) and no concurrent carcinoma in situ. This definition corresponds to a recurrence score of 0 to 1 in the European Organization for Research and Treatment of Cancer risk table (4).

Patient management. TURBT was performed under white light, and no photodynamic diagnosis technique or narrow band imaging was applied in this study. IPSI was not given when bladder perforation was suspected. After complete TURBT, an immediate single dose of pirarubicin (30 mg in 30 ml of normal saline) was administered through a Foley catheter in the operating room, and the catheter was clamped. Intravesical pirarubicin retention time was 15 min. Follow-up cystoscopy and urine cytology were performed at 3-month intervals for the first year, biannually up to 5 years, and annually thereafter. Abdominal imaging was performed depending on the situation. Recurrence was defined as the presence of macroscopic tumor at cystoscopy and a subsequent diagnosis of urothelial carcinoma with TURBT.

Clinical data acquisition. All clinical and pathological data were collected from medical records. This was a retrospective observational study and was approved by Shiga University of Medical Science Hospital (approval number 30-007).

Figure 1. Kaplan–Meier analysis of recurrence-free survival in the groups treated with transurethral resection of bladder tumor (TURBT) alone and with pirarubicin.

In vitro study. In order to obtain supportive evidence of the effectiveness of short pirarubicin instillation therapy, cellular experiments were performed as described below. Three bladder cancer cell lines, UMUC-3, T24 (American Type Culture Collection, Rockville, MD, USA) and RT112 (European Collection of Authenticated Cell Cultures, Porton Down, UK) were used. Cell culture was performed using Dulbecco's modified Eagle's medium with 10% fetal bovine serum and penicillin (100 IU/ml) streptomycin (100 µg/ml) solution (Nacalai Tesque, Kyoto, Japan) under standard culture conditions, at 37°C with 5% CO₂. Cell viability measurement was performed with the Cell Counting Kit-8 assay solution (Dojindo, Kumamoto, Japan) according to the manufacturer's instructions. Cells (3×10³ cells, 100 µl/well) were seeded in 96-well plates for 24 h, and different concentrations of pirarubicin or MMC were applied for 15 and 60 min. The cells were then thoroughly washed twice in phosphate-buffered saline and were cultured in normal medium. Three days after anticancer drug treatment, WST-8 assay was performed and 50% inhibitory concentration (IC₅₀) values were calculated.

Statistical analysis. Recurrence-free survival (RFS) was estimated using Kaplan–Meier curve and statistically analyzed (log-rank test) using EZR software (13). Hazard ratios for intravesical recurrence were calculated using the Cox hazards model. Statistical analyses of clinical data were performed using Mann–Whitney *U*-test and chi-square test. In the analyses of cellular experiments, Student's *t*-test was used. A value of *p*<0.05 was considered statistically significant.

Results

Clinical study. Characteristics of patients in the pirarubicin-treated and TURBT-only groups are shown in Table I, and clinical characteristics were not statistically different between the two groups. One- and five-year RFS were 91% and 72% for the pirarubicin-treated, and 79% and 54% for

Figure 2. Half-maximal inhibitory concentration (IC₅₀) values for pirarubicin and mitomycin C (MMC) against three bladder cancer cell lines. Data are means of triplicate experiments. Bars indicate standard deviation. *Significantly different at *p*<0.05.

the TURBT-only group (Figure 1). Pirarubicin IPSI was associated with a higher RFS than TURBT alone (log-rank, *p*=0.031). To clarify poor prognostic factors, we performed Cox hazards analysis (Table II). Larger tumor size (>10 mm) and lack of intravesical instillation therapy were revealed as significant risk factors for intravesical recurrence. Neither progression to MIBC nor adverse events regarding intravesical therapy were observed in any of the patients.

Cellular study. Figure 2 shows the IC₅₀ values obtained following 15-min and 60-min exposure of UMUC3, T24 and RT112 cells to each of the drugs. In all three cell lines, pirarubicin exerted a strong antiproliferative effect at very low concentrations. Even when the exposure time was reduced from 60 to 15 min, this tendency was consistent.

Discussion

Postoperative instillation has an important role in the prevention of intravesical recurrence of low- or intermediate-risk NMIBC (6). Especially in low-risk NMIBC, IPSI is strongly recommended (3-5). Although IPSI is listed as the standard of care in many clinical guidelines, its actual use in clinical practice varies widely across the globe (7, 14, 15). For example, clinical practice surveys have revealed that 66 and 28% of urologists in the U.S. and Europe, respectively, have never used IPSI. The low rate of adoption may be due to several factors, including individual surgeon's decisions or preferences, the surgeon's workload and educational degree, the level of nursing care, and the degree to which pharmacies are prepared (14, 15). However, as we have shown, TURBT alone is an inadequate treatment because it is associated with a significantly higher recurrence rate than is IPSI.

MMC, epirubicin and pirarubicin are all thought to be beneficial treatments (4). A recent randomized study of IPSI with MMC and pirarubicin showed no statistically difference in RFS (16). Most previous instillation studies of pirarubicin used a retention time of 1 h (8, 17). Pirarubicin was shown in the 1980s to be rapidly taken up by cultured tumor cells (10). Several clinical trials evaluating short-duration (retention time 5-15 min) instillation using pirarubicin have been carried out (11, 18). Histological analysis has revealed that installation with pirarubicin for 5 min before transurethral biopsy of bladder tumors is sufficient for adequate uptake of the drug (11). Han *et al.* reported “pirarubicin endoscopy”, in which they exploited the autofluorescence of pirarubicin to measure its uptake *via* blue light cystoscopy (18). After 15-min instillation of pirarubicin, bright red fluorescence in bladder tumors demonstrated the rapid uptake of the drug. According to these findings, we performed pirarubicin chemotherapy with a short instillation time (15 min) in the setting of IPSI for low-risk NMIBC. Our *in vitro* study using bladder cancer cell lines also supports these findings. Based on our clinical and experimental data, short-retention IPSI of pirarubicin is effective in preventing intravesical recurrence of low-risk NMIBC. In part, the complexity of postoperative care following IPSI explains its low adoption rate in clinical practice (15). A reduced retention time might benefit surgeons and other medical staff. Moreover, the duration of observation required in the recovery room could probably be shortened. Recently, intravesical gemcitabine was applied to IPSI (19-21). Although safety and tolerability of gemcitabine was reported in the IPSI setting, a longer drug dwell time (>1 h) seems to be needed to obtain an antitumor effect (20, 21).

Generally, immediate postoperative instillation induced minimal adverse events, which typically comprised chemical cystitis and skin irritation (22). However, there have been some case reports of severe adverse events due to extravasation. Oddens *et al.* reported three cases of severe complications caused by extravasation of epirubicin (23). One of these cases presented with intraperitoneal extravasation, and died from multiple organ failure associated with paralytic ileus. Filson *et al.* reported that major complications (Clavien-Dindo classification grade 3 or more) were observed in 5.2% of patients treated with perioperative MMC, with one patient needing radical cystectomy (24). Therefore, all clinical guidelines strongly recommend avoiding immediate postoperative instillation in any cases where bladder perforation is suspected (3-5). However, small non-visualized perforations of the bladder may occur even when TURBT is performed carefully. For example, Balbay *et al.* reported extravasation in 58% of patients after TURBT, even when surgeons did not suspect bladder perforation in these patients (25). Considering the above findings, shortening the intravesical retention time might be a reasonable strategy for avoiding severe

extravasation of chemotherapeutic drugs. To test this hypothesis, larger clinical studies are essential.

There are several limitations of the present study. Firstly, our cohort size was too small to derive generalizable conclusions from the findings, and the follow-up period was relatively short, especially for the MMC group. Secondly, our analysis was retrospective in nature. A prospective randomized control study is needed. Thirdly, the use of intravesical pirarubicin is not covered by health insurance in many Western countries. Despite these limitations, our results provide new insight into good practice for prevention of intravesical recurrence in patients with low-risk NMIBC. Validation of this treatment strategy will depend upon future follow-up studies.

In conclusion, almost half of patients with low-risk NMIBC encountered intravesical recurrence with TURBT alone. Short retention IPSI using pirarubicin can prevent intravesical recurrence in patients with low-risk NMIBC.

Conflicts of Interest

The Authors have no conflicts of interest directly relevant to the content of this article.

Authors' Contributions

S. Kageyama, K.K., and S. Kubota conceptualized and designed the study. T.O., Y.A., H.S., Z.N., Y.S., K.T., and C. J. K. involved in acquisition of data. C. J. K., T.C., T.Y., and A.K. involved in analysis and interpretation of data. All the Authors were involved in manuscript for all aspects of the work.

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