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ORIGINAL ARTICLE

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Prophylactic intravesical instillation chemotherapy against recurrence after a transurethral resection of superficial bladder cancer: a randomized controlled trial of doxorubicin plus verapamil versus doxorubicin alone

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Abstract *Purpose*: We investigated whether verapamil (VR), a known chemosensitizing agent of P-glycoprotein-mediated multidrug resistance, could enhance the preventative effect of doxorubicin (Adriamycin, ADM) on both intravesical recurrence and disease progression after transurethral resection (TUR) of superficial bladder cancer. *Methods*: The patients were randomized into two groups: one group received an intravesical instillation of ADM (30 mg) plus VR (15 mg) after TUR of superficial bladder cancer (19 times over 1 year), and the other group received ADM alone on the same treatment schedule. The nonrecurrence rate, the incidence of disease progression at the first recurrence and the side effects were compared over a median follow-up of 38.5 months. *Results*: Of the 226 patients registered, 157 were evaluable. No significant differences were observed in the patients' characteristics between the two groups. Although the incidence of disease progression at the first recurrence was not significantly different between the two groups, the ADM plus VR instillation group did show a significantly higher nonrecurrence rate than the ADM-only instillation group, and such significance persisted even when any possible bias was allowed for in a multivariate analysis. In terms of side effects, the incidence and severity of bladder irritation symptoms were not significantly different between the two groups. *Conclusions*: Intravesical instillation chemotherapy with ADM plus VR was found to have a significantly greater beneficial effect than with ADM alone for preventing recurrence after TUR of superficial bladder cancer.

Key words Superficial bladder cancer · Prophylaxis · Intravesical chemotherapy · Multidrug resistance

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Introduction

The high incidence of intravesical tumor recurrence following transurethral resection (TUR) remains one of the most important problems encountered in the treatment of superficial bladder cancer. To prevent such local recurrence, intravesical instillation chemotherapy with various anticancer agents is normally performed as an adjuvant to surgery. Doxorubicin (Adriamycin, ADM) has a clearly beneficial effect in preventing tumor recurrence after TUR, but its preventive effect is not necessarily sufficient and it also cannot prevent disease progression [2]. Recently, the intravesical instillation of bacillus Calmette-Guérin (BCG) has been demonstrated to be more effective than anticancer agents including ADM for preventing recurrence after TUR [5, 6]. However, this treatment has the disadvantage of causing various and frequent local and/or systemic side effects.

It has been demonstrated that chemotherapy with anthracyclines may induce P-glycoprotein-mediated multidrug resistance (MDR) in bladder cancer [10]. Cancer cells with acquired MDR are known to have an enhanced drug-efflux function [4]. In addition to such acquired MDR, intrinsic MDR associated with an increased expression of P-glycoprotein is also often observed in untreated cancers including bladder cancer [7, 10]. The expression of P-glycoprotein has been observed in from 32% to 67% of untreated primary bladder cancers [7, 10]. Therefore, overcoming MDR, whether it be aquired or intrinsic, appears to be an important factor for improving the prophylactic effect of ADM against recurrence after a TUR.

Verapamil (VR), a calcium channel blocker has been demonstrated not only to enhance the efficacy of vinca alkaloids and anthracyclines in chemosensitive tumor cells but also to overcome P-glycoprotein-mediated MDR [4, 14–16]. We have previously performed a pilot study involving intravesical therapy with ADM plus VR in patients with superficial bladder cancer, and found this treatment to be safe and effective not only for untreated cancer but also for recurrent chemoresistant superficial bladder cancer [8].

The present study was therefore conducted to examine whether VR could enhance the preventative effect of ADM on intravesical recurrence and disease progression after TUR of superficial bladder cancer. The preliminary results were reported in 1994 [9]; this report describes the final results along with an extended follow-up.

Patients and methods

As reported previously [9], the criteria of eligibility included newly diagnosed transitional cell carcinoma of the bladder, stage Ta or T1, no other active neoplasms, and no serious complications, especially no severe impairment of either hepatic or hematopoietic function. Since VR may possibly induce adverse reactions in the cardiovascular system, the absence of severe heart disease, particularly severe bradycardia, sinoatrial (S-A) block, and second- or third-degree atrioventricular (A-V) block were also included in the eligibility criteria. In addition, no patients with residual tumors due to an incomplete resection were included in this study. A tumor developing within the first postoperative month was considered to be a residual tumor, and such patients were judged to be ineligible. The patients were randomized into two groups using a central computer. Patients in group A were given a first and second intravesical instillation of ADM (30 mg in 30 ml physiological saline) immediately after and a few days after TUR, respectively. The instillations were subsequently given weekly for 2 weeks and then every 2 weeks for a further 14 weeks. After 4 months, one instillation per month was given for 8 months. Thus, a total of 19 instillations were given over a period of 1 year. Patients in group B were given an intravesical instillation of ADM (30 mg in 24 ml physiological saline) plus VR (15 mg in 6 ml solvent) according to the schedule used for the patients in group A. The patients were instructed to refrain from urinating for 2 h after each instillation. The patients were fully informed about this trial and all participated after providing full consent.

A cytologic examination of urine samples, endoscopy and routine laboratory tests (hematology test, biochemistry tests and urinalysis) were performed 4 weeks after TUR and every 3 months thereafter as a rule. A diagnosis of recurrence was established by pathological examination of either biopsy or resected specimens. The patients were checked for any local and systemic side effects, including blood pressure and heart rate, both during and after each instillation. The endpoint of this trial was set as the disease-free interval and the incidence of disease progression at the first recurrence. Therefore, the patients were followed until their first recurrence, and any treatment after recurrence was left to the discretion of each investigator. The disease-free interval was defined as the time interval between the date of operation and the time when the first recurrence was detected. When a recurrent tumor showed an increased pathological stage, that state was defined as disease progression. The median follow-up period was 38.5 months, ranging from 2 to 95.3 months.

The statistical analysis of the data was performed using the Statistical Analysis System Package. The significance of any differences in the patients' characteristics, the incidence of disease progression, and the incidence and severity of toxicity between the two groups was tested by the chi-squared test, Fisher's exact test or the *t*-test. The difference in the time to the first recurrence was assessed by the nonrecurrence curve generated by the Kaplan-Meier method and the statistical significance was analyzed by the generalized Wilcoxon test. The Cox proportional-hazard model was used to adjust for any possible bias in the background factors. Differences were considered to be statistically significant at $P < 0.05$. The annual hazard of recurrence was defined as the fraction of patients who recurred during a 1-year period. The hazard rates in the time periods were calculated using the maximum likelihood estimates from a piece-wise exponential model [3].

Results

Between July 1989 and June 1991, a total of 226 patients were enrolled in this study (Table 1). Of the 226 patients, 40 (17.7%) were considered to be ineligible based on the entry criteria: 10 patients had an invasive (pT2 or more) tumor, 10 were considered to have residual lesions, 9 had benign tumors, 7 had not primary but recurrent tumors, 3 were impossible to diagnose histologically, and 1 had an active carcinoma in another organ. A further 29 patients (12.8%) were unevaluable: 18 were lost to follow-up with inadequate treatment (less than 13 instillations), 3 withdrew due to side effects, and 8 had other protocol violations. As a result, 157 patients (69.5%) were evaluable, and no significant difference was found between the two groups in terms of the patients' characteristics (Table 2).

The overall 3-year nonrecurrence rate was 64.5% in group A and 78.4% in group B, respectively, and group B showed a significantly higher nonrecurrence rate than group A ($P = 0.0303$, generalized Wilcoxon test; Fig. 1). To adjust for any possible bias due to an imbalance of the background factors, the Cox proportional-hazard model was used. As shown in Table 3, three (tumor growth pattern, number of tumors and histological grade) out of seven background factors were found to significantly influence the nonrecurrence rate in

Table 1 Disposition of the subjects

	Group A	Group B	Total (%)
Enrolled	113	113	226 (100)
Eligible	92	94	186 (82.3)
Evaluable	76	81	157 (69.5)

Table 2 Characteristics of the evaluable patients

Factor	Group A (n = 76)	Group B (n = 81)	P-value
Age (mean ± SD)	61.5 ± 12.2	65.4 ± 12.3	0.0507
Sex			
M	54	67	0.0820
F	22	14	
Growth pattern			
Papillary, pedunculated	56	60	0.5540
Papillary, sessile	17	19	
Nonpapillary, pedunculated	0	1	
Nonpapillary, sessile	3	1	
Tumor size (cm)			
<1	29	29	0.5510
1–3	40	41	
3–5	7	9	
≥5	0	2	
Number of tumors			
1	56	48	0.1610
2–4	14	23	
≥5	6	10	
Stage			
pTa	52	53	0.6910
pT1	24	28	
Grade			
G1	28	26	0.7060
G2	42	46	
G3	6	9	

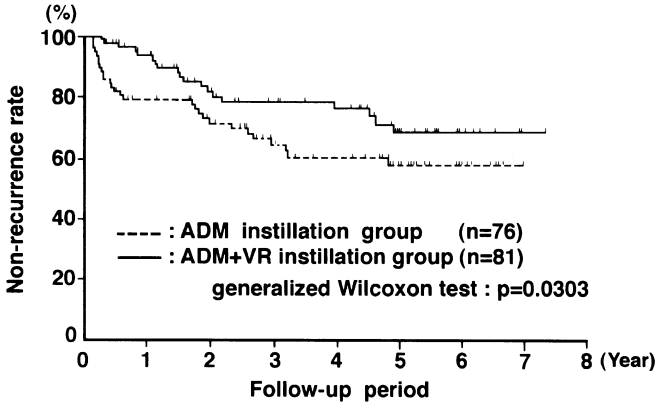

Fig. 1 Comparison of nonrecurrence rates in the ADM-only instillation group versus the ADM plus VR instillation group

a univariate analysis. As a result, the significance of the difference between groups A and B was adjusted for these three factors in the multivariate analysis. As shown in Table 4, the significance persisted when the nonrecurrence rate was adjusted for the three background factors together.

Figure 2 demonstrates the hazard of recurrence for each 1-year period after a TUR. In group A, the peak hazard of recurrence (0.0006) occurred in the year immediately after a TUR with a slowly decreasing hazard of recurrence. In group B, however, such a peak hazard was remarkably prevented, and the hazard rate of recurrence remained low throughout the observation period. The hazard rate dropped to 0 by year 5 (no patient recurred after year 5) in either group.

The incidence of tumor progression in recurrent tumors is presented in Table 5. Of 28 recurrences in group A, tumor progression occurred in 6 (21.4%) including 2 recurrences with the pT2 tumor (7.1%), whereas it occurred in 3 (15.8%) of 19 recurrences in group B. No tumors with muscle invasion developed in any of the recurrences in group B, and no significant difference was

Table 3 Univariate analysis of the factors influencing recurrence

Variable	Hazard ratio	95% confidence interval	P-value
Age	1.011	0.986–1.036	0.4011
Sex (M/F)	1.039	0.529–2.043	0.9127
Growth pattern (Papillary, pedunculated/papillary, sessile/nonpapillary, pedunculated/nonpapillary, sessile)	1.757	1.322–2.333	0.0001
Tumor size (<1/1–3/3–5/≥5 cm)	1.065	0.696–1.629	0.7702
Number of tumors (<1/2–4/≥5)	1.720	1.207–2.450	0.0027
Stage (pTa/pT1)	1.243	0.719–1.761	0.4683
Grade (G1/G2/G3)	1.794	1.122–2.868	0.0146

Table 4 Multivariate adjusted hazard ratios for recurrence

Variables and models (Group A and group B)	Hazard ratio[a]	95% confidence interval	*P*-value
Univariate	0.591	0.330–1.058	0.0766
Adjusted for growth pattern (Papillary, pedunculated/papillary, sessile/nonpapillary, pedunculated/nonpapillary, sessile)	0.573	0.320–1.027	0.0583
Adjusted for number of tumours (1/2–4/⩾5)	0.507	0.278–0.924	0.0021
Adjusted for grade (G1/G2/G3)	0.570	0.318–1.021	0.0556
Adjusted for growth pattern, number of tumors and grade	0.523	0.276–0.991	0.0382

[a]A hazard ratio below 1.0 indicates that group B has less risk than group A

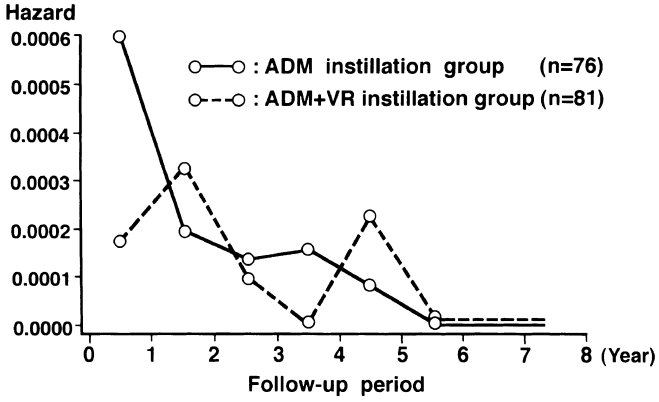

Fig. 2 Annual hazard rate of recurrence for the ADM-only instillation group versus the ADM plus VR instillation group

Table 5 Incidence of tumor progression in recurrent tumors

	Number of cases (%)		*P*-value
	Group A (n = 28)	Group B (n = 19)	
Progression	6 (21.4)	3 (15.8)	0.7200
pTa→pT1	4 (14.3)	3 (15.8)	
pT1→pT2	2 (7.1)	0	
No progression	22 (78.6)	16 (84.2)	

observed in the frequency of tumor progression between the two groups.

The side effects were evaluated in 218 patients (96.9%), excluding 8 who received fewer than three instillations. The incidence and severity of the side effects are presented in Table 6. As a local side effect, bladder irritation symptoms (polakisuria and/or pain on urination) were observed in 14.8% and 19.1% of groups A and B, respectively. Three patients (2.7%) in group B withdrew from the study after the sixth, ninth and tenth instillation, respectively. However, there was no significant difference in the incidence and severity of the bladder irritation symptoms between the two groups. No systemic or hematological side effects were observed in either group.

Discussion

The present study was conducted to examine whether VR, a known chemosensitizing agent against P-glycoprotein-mediated MDR, could enhance the prophylactic effect of ADM, an MDR-related agent, on intravesical recurrence and tumor progression after TUR of superficial bladder cancer. The dose of ADM and the duration of instillation were set at 30 mg per 30 ml and 19 instillations over a 1-year period, respectively, according to our previous experience, in which this regimen provided a high nonrecurrence rate without any significant side effects when the instillation was started immediately after TUR [17]. Niijima et al. [11] have also reported tumor recurrence to be significantly inhibited with intravesical instillation of ADM at this concentration 21 times over a 2-year period after TUR. The dose of VR was set at 15 mg per 6 ml, which was the maximal clinical dose for an intravenous bolus injection, and it was instilled together with 30 mg ADM dissolved in 24 ml physiological saline. Under these conditions, the

Table 6 Incidence and severity of side effects

Side effect		Group A (%) (n = 108)	Group B (%) (n = 110)	*P*-value
Bladder irritation symptoms[a]	–	90 (83.3)	89 (80.9)	0.3850
	+	9 (8.3)	7 (6.4)	
	++	9 (8.3)	11 (10.0)	
	+++	0	3 (2.7)	
Urethral discomfort or pain[a]	–	107	105 (95.5)	0.3700
	+	1 (0.9)	3 (2.7)	
	++	0	2 (1.8)	
	+++	0	0	

[a]– none, + mild (intravesical chemotherapy could be completed without any treatment), ++ moderate (intravesical chemotherapy could be completed with appropriate treatment), +++ severe (intravesical chemotherapy was discontinued)

VR concentration was considered to be more than 50 times higher than that needed to enhance the drug sensitivity or overcome the drug resistance of malignant tumors in vitro [14–16]. Before starting this study, we confirmed that the intravesical instillation of ADM plus VR at these concentrations produced a favorable response against superficial bladder cancer without any significant side effects [8]. In comparison with the untreated primary cases, the complete response rate was substantially higher in the patients with recurrence who had previously received prophylactic intravesical instillation chemotherapy including ADM.

The instillation of ADM in the presence or absence of VR was started immediately after TUR in order to increase the prophylactic effect [17]. Therefore, tumors were judged to be cystoscopically superficial and patients were required to be registered without any information regarding the histology of the TUR specimens. Consequently, quite a few patients were found to be ineligible after registration, and the prophylactic effect of ADM in the presence or absence of VR was evaluated in 157 (69.5%) of the 226 enrolled patients. Nevertheless, no significant difference was seen in the patients' characteristics between the two groups, and the patient groups were considered to be well matched. Furthermore, in the statistical analysis, the Cox proportional-hazard model was used to adjust the delicate bias.

We focused our attention on the first recurrence after TUR and did not perscribe any treatment after the first recurrence. The ADM plus VR instillation group showed a significantly higher nonrecurrence rate than ADM-only instillation group, and this significance persisted even when possible bias was allowed for in a multivariate analysis. These results clearly indicate that VR can potentiate the prophylactic effect of ADM against recurrence after TUR of superficial bladder cancer.

Intravesical recurrence after TUR of superficial bladder cancer is considered to occur by the following mechanisms: (1) the implantation of tumor cells into the bladder wall, (2) the development of microlesions or precancerous lesions, (3) the growth of a residual tumor at the time of TUR, and (4) the development of a new tumor [1]. Based on a profile of the hazard of recurrence, Akaza [1] reported that tumor recurrences of types (1) to (3) may occur at 3 to 12 months after TUR (early recurrence), and also demonstrated that intravesical chemotherapy can reduce the risk of such early recurrence. In this study, such an early peak hazard of recurrence was still observed in the ADM-only instillation group. If the prophylactic instillation of ADM is not performed, a much higher peak hazard of recurrence might be observed in the first year after TUR. In the ADM plus VR instillation group, however, no such peak hazard of recurrence was observed in the first year after TUR and the hazard rate remained low throughout the observation period. This clearly indicates that the higher nonrecurrence rate in the ADM plus VR instillation group was due to an increased preventative effect against early recurrence after TUR.

Tumor progression was seen in 15.8% of the ADM plus VR instillation group, which was somewhat lower than the 21.4% found in the ADM-only instillation group, but this difference was not statistically significant. These values are in close agreement with the tumor progression rates in the recurrences of patients treated with intravesical chemotherapy reported by Akaza et al. [2] and Schulman et al. [13]. Our results thus suggest that the instillation of VR does not influence the occurrence of tumor progression. However, it may be noteworthy that no tumors with muscle invasion developed in any of the recurrences in the ADM plus VR instillation group.

In terms of toxicity, the incidence and severity of the side effects did not significantly differ between the two groups. The incidence of bladder irritation symptoms in the two groups was almost the same as that reported by Obata et al. [12] who performed ADM instillation (20 mg in 40 ml physiological saline) using almost the same schedule as ours. In the previous pilot study [8], we confirmed that the absorption of VR into the systemic circulation is minimal when 15 mg of VR is instilled into the bladder with 30 mg of ADM. No systemic or hematological side effects were observed in either group, which is also in accordance with the findings of the previous pilot study [8]. These results therefore indicate that the prophylactic intravesical instillation of ADM plus VR is safe even when the instillation is started immediately after TUR.

In conclusion, based on the above findings, the intravesical instillation of ADM plus VR is considered to be safe and more effective than the instillation of ADM alone in preventing intravesical recurrence, particularly early recurrence, after TUR of superficial bladder cancer. A prospective randomized trial is warranted to compare intravesical ADM plus VR with BCG, which has been demonstrated to be more effective than ADM in preventing recurrence after TUR and has thus now become the method of choice for recurrence prophylaxis [5, 6].

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