


Original Article

Intravesical mitomycin C (MMC) and MMC + cytosine arabinoside for non-muscle-invasive bladder cancer: a randomised clinical trial

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Abstract Objectives

To compare the urinary pH, recurrence-free survival (RFS), and safety of adjuvant intravesical therapy in patients with non-muscle-invasive bladder cancer (NMIBC) receiving mitomycin C (MMC) therapy and MMC + cytosine arabinoside (Ara-C) therapy.

Patients and Methods

A total of 165 patients with NMIBC from six hospitals were randomly allocated to two groups: weekly instillation of MMC + Ara-C (30 mg/30 mL + 200 mg/10 mL) for 6 weeks and the same instillation schedule of MMC (30 mg/40 mL). The primary outcome was RFS, and secondary outcomes were urinary pH and toxicity in the two groups.

Results

A total of 81 and 87 patients were randomised into the MMC and MMC + Ara-C groups, respectively. Overall, the RFS in the MMC + Ara-C group was significantly longer ($P = 0.018$) than that in the MMC group. A similar significant difference was detected in patients with intermediate-risk NMIBC, but not in those with high-risk NMIBC. The mean (SD) urinary pH was significantly higher in the MMC + Ara-C group than in the MMC group, at 6.56 (0.61) vs 5.78 (0.64) ($P < 0.001$), and the frequency of a urinary pH of >7.0 in the MMC and MMC + Ara-C groups was 6.3% and 26.7%, respectively ($P < 0.001$). Multivariate analysis models including clinicopathological features and second transurethral resection demonstrated that increased urinary pH was associated with better outcomes (hazard ratio 0.18, 95% confidential interval 0.18–0.038; $P < 0.001$). In all, there were 14 and 10 adverse events in the MMC and MMC + Ara-C groups, respectively, without a significant difference ($P = 0.113$).

Conclusions

Our randomised clinical trial suggested that intravesical therapy with MMC and Ara-C is useful and safe for patients with intermediate-risk NMIBC. Increase in urinary pH with Ara-C is speculated as a mechanism for increased anti-cancer effects.

Keywords

intravesical therapy, mitomycin C, cytosine arabinoside, urinary pH, non-muscle-invasive bladder cancer, randomised trial, #BladderCancer, #blcsm, #uroonc

Introduction

The most representative characteristic of non-muscle-invasive bladder cancer (NMIBC) is the high frequency of disease relapse after transurethral resection (TUR), despite complete tumour resection. Therefore, various guidelines recommend adjuvant intravesical therapy after TUR [1–3].

Chemotherapeutic agents including mitomycin C (MMC) and BCG are commonly used for intravesical therapy according to pathological features and patient conditions. In general, the anti-cancer effects of intravesical therapy using BCG are greater than those of other chemotherapeutic agents.

Therefore, intravesical BCG therapy is recommended for patients with intermediate- to high-risk NMIBC [1–3]. However, the high frequency of adverse effects is a critical disadvantage of intravesical BCG therapy [4]. Additionally, the anti-cancer effects of intravesical BCG therapy are desirable because up to 70% of patients experience recurrence and some recurrent tumours progress in high-risk cases [5,6]. Therefore, detailed information on more effective and safe regimens of intravesical therapy is essential to improve the prognosis of patients with NMIBC.

Various intravesical therapies, such as BCG-based therapy, combination therapy with chemotherapeutic agents, and newly developed agent-based or device-used therapies, have been suggested, and their anti-cancer effects and toxicities have been investigated in clinical trials [7–11]. However, BCG-based therapies are disadvantageous in terms of adverse events (AEs), and newly developed methods are usually expensive and require special equipment. Moreover, the approval status of agents and devices varies among countries.

Mitomycin C is often used as an intravesical therapy after TUR; however, the anti-cancer effect of conventional intravesical MMC therapy may not be satisfactory in patients with NMIBC, especially in intermediate- to high-risk disease. Although hyperthermic intravesical MMC therapy is reported to increase anti-cancer effects without additional toxicity in *in vivo* and *in vitro* studies [9,12,13], it requires special devices.

In the present study, we focussed on the close association of the pharmacokinetics of intravesical MMC therapy with urinary pH considering that its anti-cancer effects were remarkably increased after urine alkalinisation [14–16]. In fact, urine alkalinisation during intravesical MMC therapy by oral sodium bicarbonate administration was performed to prevent the recurrence and progression of NMIBC [17,18]. However, the administration of sodium bicarbonate is associated with the risk of hypernatraemia [19,20]. Therefore, we searched for methods with higher certainty and safety that would increase urinary pH. Finally, we used cytosine arabinoside (Ara-C) to enhance the anti-cancer effects of MMC in adjuvant intravesical therapy after complete TUR for three reasons:

(i) Ara-C may be useful in urine alkalinisation because its pH is 8.0–9.3 according to the product label, (ii) Ara-C has been used for intravesical therapy to prevent bladder cancer recurrence, and (iii) safety of intravesical therapy with MMC + Ara-C has been previously reported [21,22]. Briefly, we hypothesised that combination with Ara-C would increase the anticancer effects of MMC after urine alkalinisation and provide additive anti-cancer effects.

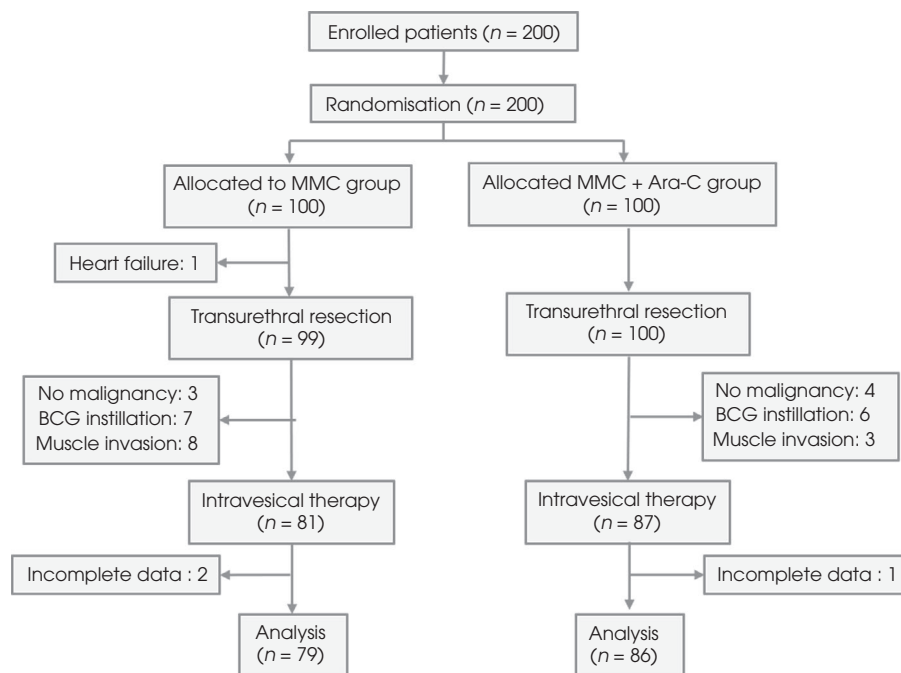
Patients and Methods

Study Design and Participants

This was a prospective randomised controlled trial conducted between June 2012 and March 2017, and 200 patients with clinical NMIBC were recruited from six institutes in Japan. This protocol was approved by the Institutional Review Board (IRB) of Nagasaki University Hospital (No. 16052301) and was registered on the University hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN000008173). The eligibility criterion was histologically diagnosed NMIBC (pTis, pTa, or pT1) treated with complete TUR. The exclusion criteria were: i) WHO performance status of ≥ 3 , ii) contraindication of drugs, iii) other malignancies and additional treatment plans, iv) disadvantages for patients due to outpatient care, v) no histological diagnosis of urothelial cancer, vi) no written informed consent, and vii) aged < 20 years. Disease risk was divided into three groups: low-, intermediate-, and high-risk according to the published guidelines [2]. At first, all patients with NMIBC with complete TUR were recruited for this trial. However, since 2015, a single, immediate, post-TUR instillation of MMC and intravesical BCG therapy has been strongly recommended for low- and high-risk patients, respectively, due to the revision of The Japanese Urological Association Guideline. In fact, this recommendation was clearly mentioned in the explanation and consent forms. Participant enrolment was performed before TUR for the following reasons. At our institutes, histological diagnosis is performed 3–4 weeks after surgery and intravesical therapy is usually initiated 4 weeks after complete TUR. Our IRB encourages that our protocol includes sufficient time for decision making. Therefore, in our study, we performed randomisation before TUR.

Randomisation

Randomisation was performed using the envelope method, and patients were allotted to either the MMC or MMC + Ara-C group in a 1:1 ratio. The flow chart and study design of patient enrolment, allocation, therapy, and analysis are shown in Figure 1. In this trial, the study population was not stratified by factors such as the treating centre and carcinoma *in situ* (CIS). Prior to enrolment, the standard methods and their predicted anti-cancer effects, AEs, and risks were explained to the patients.

Fig. 1 Flow chart of enrolment, allocation, therapy, and analysis.

Regimens

All patients in the MMC and MMC + Ara-C group received a single instillation of MMC (30 mg/40 mL) immediately after TUR. Intravesical therapy was initiated 4–6 weeks after complete TUR, and weekly instillation was scheduled for 6 weeks at a dose of 30 mg MMC in 40 mL physiological saline or 30 mg MMC in 30 mL physiological saline combined with 200 mg/10 mL Ara-C with retention for 1 h. Maintenance treatment after the 6-week instillation period was not implemented in either group. The test drugs were administered after the bladder was completely emptied using a urethral catheter. Urinary pH was measured in the first voided urine after drug instillation using the same pH meter (B-71X, HORIBA Scientific, Kyoto, Japan).

Outcomes

The primary outcome was comparison of recurrence-free survival (RFS) periods. Secondary outcomes were urinary pH and toxicities in our two trial groups. Cystoscopy was performed every 3 months for the first 3 years, every 6 months for the next 5 years, and yearly thereafter depending on pathological features and activities of daily living. Urine cytology was also evaluated using a similar schedule as cystoscopy. Ultrasonography, CT, or MRI was performed as necessary; however, ultrasonography was performed annually for at least 5 years. AEs were evaluated using the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

Statistical Analyses

The Student's *t*-test and chi-square test were used for comparisons of continuous and categorical data, respectively. The RFS period was compared between the MMC and MMC + Ara-C groups using Kaplan–Meier curves and the log-rank *p* test. In Cox regression analysis, results were expressed as hazard ratios (HRs) with 95% CIs, and *P* values. The coefficient of variation was calculated by SD/mean to evaluate the variation in urinary pH according to the regimens. All statistical analyses were performed using the statistical package StatView for Windows (version 5.0, Abacus Concept, Inc., Berkeley, CA, USA).

Results

In a preliminary study, the pH of solutions including MMC (30 mg/40 mL physiological saline), Ara-C (200 mg/10 mL), and MMC + Ara-C (30 mg + 200 mg/40 mL) solution in each of the 10 samples was measured. The Ara-C solution was more alkaline (mean [SD] pH 9.05 [0.23]) than the MMC solution (mean [SD] pH 7.54 [0.31]), and the pH of the MMC + Ara-C solution (mean [SD] pH 8.86 [0.31]) was remarkably higher ($P < 0.001$) than that of the MMC solution (Table 1). In this study, all patients in both groups tolerated the 1-h dwell time and full induction treatment well.

A total of 200 patients were randomised into the MMC (100) or MMC + Ara-C (100) groups after diagnosis of clinical NMIBC. After exclusion due to heart failure, pathological

Table 1 pH of each experimental solution and urinary pH in study population.

	MMC	Ara-C	MMC + Ara-C	P
Experimental solution				
Sample 1	7.800	9.100	8.900	–
2	8.300	9.200	9.000	–
3	7.300	9.000	9.000	–
4	8.500	9.100	9.000	–
5	8.200	9.200	9.200	–
6	6.800	8.500	8.400	–
7	7.200	9.300	9.000	–
8	7.500	9.200	9.000	–
9	7.200	9.000	8.900	–
10	6.800	8.900	8.200	–
Mean (SD)	7.56 (0.61)	9.05 (0.23)	8.86 (0.3)	<0.001*
Urine samples of patients				
Mean (SD)	5.78 (0.64)	–	6.56 (0.61)	<0.001
Coefficient of variation	0.111	–	0.092	–
pH ≥ 5.5 ; n (%)	53 (67.1)	–	82 (96.5)	<0.001
≥ 6.0	27 (34.2)	–	71 (83.5)	<0.001
≥ 6.5	16 (20.3)	–	49 (57.0)	<0.001
≥ 7.0	5 (6.3)	–	23 (26.7)	<0.001

*MMC + Ara-C vs MMC.

diagnosis, choice of BCG therapy, and incomplete data, 79 and 86 participants were treated with MMC and MMC + Ara-C therapy, respectively. Differences in the clinicopathological features between the MMC and MMC + Ara-C groups were non-significant (Table 2). Additionally, the rates of patients with low-, intermediate-, and high-risk disease were similar between the two groups ($P = 0.504$). Although a second TUR was performed in 50 patients (30.3%), the rate was similar between the MMP (34.2%) and MMC + Ara-C groups (26.7%, $P = 0.299$). As mentioned before, the standard treatment strategies for NMIBC were explained before enrolment. Briefly, we recommended intravesical BCG therapy to patients with high-risk disease. Therefore, the number of patients with concomitant CIS or large tumours (>3 cm) was relatively low (6.1% and 3.6%, respectively), and the number of patients with low-risk tumours was 22 (13.3%). Finally, 68 high-risk patients (41.2%) wished to participate due to high frequency and/or severe AEs of intravesical BCG therapy. In all, 42 (25.5%) had recurrent tumours at enrolment; however, all of them were not previously treated with intravesical BCG or intravesical MMC therapy. On the other hand, 14/17 (82.4%) and 21/25 (84.0%) recurrent tumours in the MMC and MMC + Ara-C groups were previously treated with intravesical therapy using other chemotherapeutic agents such as epirubicin and doxorubicin.

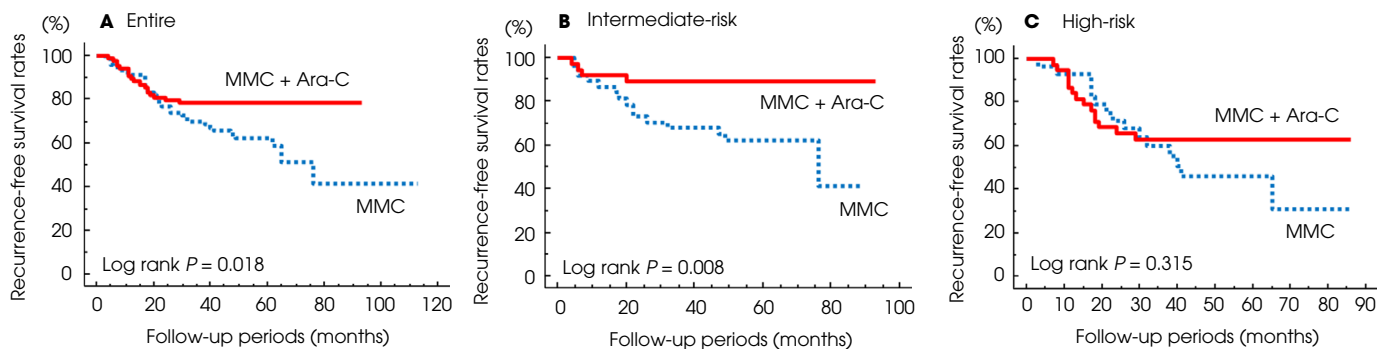
As shown in Table 1, urinary pH in patients who received MMC + Ara-C therapy (mean [SD] pH 6.56 [6.12]) was significantly higher ($P < 0.001$) than that in patients who received MMC therapy (mean [SD] pH 5.78 [0.64]). However, although almost all patients in the MMC + Ara-C group (96.5%) had a urinary pH of ≥ 5.5 , the proportion of MMC-group patients with urinary pH of ≥ 5.5 was 67.1%

($P < 0.001$; Table 1). Similarly, the number of patients with urinary pH of ≥ 6.0 and ≥ 6.5 in the MMC + Ara-C group (83.5% and 57.0%, respectively) was significantly higher ($P < 0.001$) than that in the MMC group (pH ≥ 6.0 : 83.5% vs 34.2% and pH ≥ 6.5 : 57.0% vs 20.3%). Additionally, $\geq 25\%$ of the patients who received intravesical MMC + Ara-C therapy had a pH of ≥ 7.0 ; however, the frequency of pH of ≥ 7.0 in the MMC group was only 6.3% ($P < 0.001$; Table 1). The coefficient of variation of urinary pH in the MMC group (0.111) was >1.21 times wider than that in the MMC + Ara-C group (0.092; Table 1). This result indicates that urinary pH in the MMC + Ara-C group was higher and more stable than that in the MMC group.

The mean (SD) and median (interquartile range) follow-up periods were 46.4 (23.7) and 52 (24–62) months, respectively. The mean follow-up period in the MMC + Ara-C group (48.7 months) tended to be higher than that in the MMC group (43.8 months); however, the difference was not significant ($P = 0.177$). In survival analyses, Kaplan–Meier survival curves showed that the RFS period in the MMC + Ara-C group was significantly longer ($P = 0.018$) than that in the MMC group for all patients (Fig. 2A). Next, when similar analyses were performed in patients with intermediate- and high-risk disease, except for low-risk tumours because recurrence occurred in just one patient, a significant relationship was observed in patients with intermediate-risk disease ($P = 0.008$; Fig. 2B), but not in those with high-risk disease ($P = 0.315$; Fig. 2C). Actually, the RFS rate at 24 months for patients with intermediate-risk disease was obviously higher in the MMC + Ara-C group than in the MMC group (88.9% vs 70.6%); such a wide difference was not observed for patients with high-risk disease. On the other hand, when a similar analysis was

Table 2 Clinicopathological features.

	Total 165 (100)	MMC 79 (47.0)	MMC + Ara-C 86 (53.0)	P
Age, years, mean (SD)	72.7 (9.2)	73.3 (9.4)	72.1 (9.1)	0.424
Gender, <i>n</i> (%)				
Male	130 (78.8)	61 (77.2)	69 (80.2)	0.636
Female	35 (21.2)	18 (22.8)	17 (29.8)	
Grade, <i>n</i> (%)				
Low	80 (48.5)	41 (51.9)	39 (45.3)	0.400
High	85 (51.5)	38 (48.1)	47 (54.7)	
pT stage, <i>n</i> (%)				
Ta	118 (71.5)	59 (74.7)	59 (68.6)	0.388
T1	47 (28.5)	20 (25.3)	27 (31.4)	
Concomitant CIS, <i>n</i> (%)				
Presence	6 (3.6)	4 (5.1)	2 (2.3)	0.348
Multiplicity, <i>n</i> (%)				
Multiple	83 (50.3)	35 (44.3)	48 (55.8)	0.140
Recurrent tumour, <i>n</i> (%)				
Yes	42 (25.5)	17 (21.5)	25 (29.1)	0.266
Tumour size, <i>n</i> (%)				
>3 cm	10 (6.1)	6 (7.6)	4 (4.7)	0.429
Risk stratification, <i>n</i> (%)				
Low	22 (13.3)	12 (15.2)	10 (11.6)	0.504
Intermediate	75 (45.4)	38 (48.1)	37 (43.0)	
High	68 (41.2)	29 (40.8)	39 (45.3)	

Fig. 2 Survival curves according to risk stratification in our study population.

performed across all patients, including those that had been excluded (intent-to-treat population; $n = 200$), RFS was significantly longer in the MMC + Ara-C group than in the MMC group ($P = 0.037$) (Figure S1). Survival analyses according to disease risks were not performed because participants with no malignancy or muscle-invasive disease were included in the study cohort. Regarding disease progression after intravesical therapy, four of 79 patients (5.1%) in the MMC group showed metastasis (three) and muscle-invasive disease (one), while two of 86 patients (2.3%) in the MMC + Ara-C group developed metastasis.

As described above, a clinical benefit of MMC + Ara-C therapy was observed for patients with intermediate-risk NMIBC. Therefore, detailed analyses of the influence of urinary pH on the inhibition of recurrence by MMC + Ara-C instillation were performed in patients with intermediate-risk NMIBC. Univariate Cox proportional hazard analysis also

showed urinary pH to be negatively associated with recurrence risk in patients with intermediate-risk disease (HR 0.17, 95% CI 0.08–0.34; $P < 0.001$; Table 3). A similar univariate analysis demonstrated that recurrence risk was reduced to one-quarter with the MMC + Ara-C therapy (HR 0.25, $P = 0.015$; Table 3). Based on these univariate analyses results, independent roles of urinary pH or intravesical MMC + Ara-C therapy for RFS in patients with intermediate-risk NMIBC were investigated with multivariate analyses model including sex, age, and second TUR (Table 3). Both urinary pH and intravesical MMC + Ara-C therapy were recognised as independent factors (HR 0.12, 95% CI 0.05–0.29, $P < 0.001$; and HR 0.24, 95% CI 0.08–0.79, $P = 0.019$, respectively) for RFS (Model A). Next, when the independent role of MMC + Ara-C instillation and urinary pH was analysed (Model B), urinary pH was significantly associated with RFS (HR 0.18, 95% CI 0.08–0.38; $P < 0.001$),

Table 3 Impact on recurrence in patients with intermediate-risk NMIBC.

	Urinary pH		MMC + Ara-C instillation	
	HR (95% CI)	P	HR (95% CI)	P
Univariate	0.17 (0.08–0.34)	<0.001	0.25 (0.08–0.76)	0.015
Multivariate				
Model A	0.12 (0.05–0.29)	<0.001	0.24 (0.08–0.79)	0.019
Model B	0.18 (0.08–0.38)	<0.001	0.58 (0.19–1.78)	0.344
Model C	0.12 (0.05–0.32)	<0.001	0.83 (0.21–3.25)	0.794

Model A, adjusted by gender, age, second transurethral resection; Model B, adjusted by urinary pH or MMC + Ara-C therapy; Model C, adjusted by gender, age, second TUR, and urinary pH or MMC + Ara-C therapy.

but MMC + Ara-C instillation was not (HR 0.58, $P = 0.344$; Table 3). Additionally, in the multivariate analysis model of sex, age, second TUR, and urinary pH or MMC + Ara-C therapy (Model C), urinary pH was identified as an independent predictor of RFS (HR 0.12, 95% CI 0.05–0.32; $P < 0.001$).

In all patients, 14 and 10 AEs occurred in the MMC and MMC + Ara-C groups, respectively (Table S1). The frequency of local AEs in the MMC group (15.2%) was higher than that in the MMC + Ara-C group (7.0%); however, the frequency of systemic events was higher in the MMC + Ara-C group than in the MMC group (4.7% vs 2.5%; Table S1). Finally, the differences in the frequency of AEs between the two groups were non-significant ($P = 0.113$). Four AEs (micturition pain, haematuria, back pain, and skin rash) affected the instillation schedule in the MMC group, whereas three AEs (haematuria, urethral pain, and skin rash) caused delays in the MMC + Ara-C group. Only one patient (in the MMC group) required additional medication for a skin rash. However, there were no severe AEs (CTCAE ≥ 3). No abnormal laboratory data were observed for any of the patients.

Discussion

One of the most unique characteristics of the present study is that Ara-C was used as an alkalisating agent in the intravesical therapy. In our preliminary study, the mean urinary pH of the MMC + Ara-C group was higher than that of the MMC group. Regarding the associations between pH and pharmacological activities of MMC, several investigators have demonstrated that urine alkalisating increases tumour exposure of MMC via regulation of its degradation and absorption, and such findings have led to the enhancement of its anti-cancer effects [14–16]. A retrospective study demonstrated urinary pH to be closely associated with tumour recurrence in patients with NMIBC treated with intravesical MMC therapy [23]. Additionally, regarding MMC stability in urine, an *in vitro* study demonstrated that MMC degradation at pH 5.0 (half-life, 2.8 h) proceeded faster than

that at pH 6.0 (half-life, 12.2 h), and both these degradations were remarkably higher than that at pH 7.0 (half-life, 38.0 h) [15]. Furthermore, another *in vitro* study demonstrated the mean rates of active MMC in buffer solutions of pH 5.0, 6.0, 7.0, and 8.0 as 47.4%, 64.2%, 95.4%, and 100.0%, respectively [18]. Additionally, this study demonstrated that the 50% inhibitory concentration of cancer cell survival in the buffer of pH ≥ 6.5 was remarkably lower than that in the buffer of pH < 6.0 (e.g. 1.33 vs 1.98 $\mu\text{g/mL}$ in T24 cells and 0.92 vs 2.3 $\mu\text{g/mL}$ in 253 J cells) [18]. Thus, that study suggested that the rate of active MMC is positively associated with urinary pH, and urinary pH of ≥ 6.5 is speculated to be essential in maintaining the anti-cancer effects of intravesical MMC therapy. In the present study, the frequency of urinary pH of ≥ 6.5 was more than three-times higher in the MMC + Ara-C group than in the MMC group (83.7% vs 26.6%), and that of urinary pH of ≥ 7.0 was more than four-times higher in the MMC + Ara-C group than in the MMC group (26.7% vs 6.3%). Based on these findings, we propose that the anti-cancer effects of MMC were maintained as much as possible by combination with Ara-C.

In addition to *in vitro* studies, increased urinary pH was reported to be closely associated with decreased recurrence in 124 patients with NMIBC treated with intravesical MMC therapy in multivariate analysis models [23], and our present results are in accordance with theirs. However, considering the mechanisms of the anti-cancer effects of intravesical MMC therapy according to urinary pH, the influence of urinary pH on the risk of recurrence in patients with NMIBC without MMC instillation must be well understood. A previous report had demonstrated urinary pH as not being significantly associated with recurrence in 252 patients with NMIBC undergoing surveillance after TUR [24]. In short, that study demonstrated that the median time of recurrence in patients with urinary pH of > 6 was similar to that of patients with urinary pH of ≤ 6 , and no significant relationship was observed in either low- or high-grade disease [24]. From these facts, we speculate that the enhancement of anti-cancer effects by urine alkalisating is modulated via the upregulation of MMC activity, but not directly by urinary pH increase.

Information on the anti-cancer effects of intravesical MMC + Ara-C therapy after TUR in NMIBC is limited; however, one report showed that the cumulative recurrence rates at 1 and 3.5 years after TUR in patients with NMIBC were 16.7% and 41.9%, respectively [21]. Unfortunately, in that study, data on RFS according to risk stratification were not shown. However, we noticed that their recurrence rates were similar to our present results in patients with high-risk tumours, although their treatment strategies and medical equipment were different because their study was reported in 1982 [19]. On the other hand, there was a randomised study on the prevention of recurrent bladder cancer by intravesical

MMC + Ara-C therapy after surgery for upper tract urothelial cancer (UTUC) [22]. That study randomised 27 patients with UTUC into MMC (20 mg) + Ara-C (200 mg) and non-instillation groups, and intravesical therapy was administered 28 times over 2 years [22]. As a result, their regimen suppressed recurrence of bladder tumour without severe AEs. Although the anti-cancer effects of their study cannot be compared with our present results because the methodology and patient backgrounds are different, their results suggested that intravesical instillation of MMC + Ara-C is a useful and safe regimen for suppressing recurrence.

In addition to urinary pH, urine production, residual urine volume, MMC dose, dosing volume, and dwell time can affect the pharmacological effects of MMC [25]. In that study, MMC dose, dosing volume, and dwell time were similar among all hospitals, and test drug(s) were administered after complete emptying of the bladder. However, data regarding urine production were not available for our present study population. This is a limitation of the present study. A major limitation is that the MMC dose (30 mg) used was not the standard dose according to American and European guidelines. In short, in many countries, 20 or 40 mg MMC is usually used for intravesical therapy. However, there is no definite consensus on whether 20 or 40 mg should be used. Actually, according to the universal public insurance system in Japan, our institutes used 20, 30, or 40 mg of MMC for intravesical therapy, with the most commonly used dose being 30 mg. Therefore, we selected 30 mg MMC for the present trial. A main aim of the present study was to clarify the enhancement of anti-cancer activity of intravesical MMC therapy based on increased urinary pH with the Ara-C combination solution. We believe that our present results addressed this important issue because the MMC dose was the same in both groups. Furthermore, the number of patients was relatively low. Therefore, further clinical trials using 20 or 40 mg MMC with a larger study population are necessary to determine the clinical usefulness of intravesical MMC + Ara-C therapy in patients with NMIBC. In the present study, treatment strategies with intravesical therapy are discussed, especially in patients with intermediate-risk NMIBC [26,27]. We believe that our present results provide important information for discussion on this issue. However, we would like to mention that our regimens would not be considered optimal in some respects, such as the MMC dose and maintenance period. Therefore, modification of MMC + Ara-C instillation is necessary to improve the anti-cancer effects in patients with NMIBC. We also suggest the importance of further studies on the suppressive effects of MMC + Ara-C therapy on tumour progression in patients with NMIBC because this frequency in the MMC + Ara-C group had a trend of being lower compared to the MMC group.

In conclusion, our randomised clinical trial demonstrated that intravesical therapy with MMC plus Ara-C had greater

inhibitory effects on recurrence than did MMC alone in patients with intermediate-risk NMIBC. For this finding, increased urinary pH by adding Ara-C was speculated as a mechanism. Regarding toxicities, differences between the two intravesical therapies were non-significant. This combined regimen is recommended for patients with NMIBC, especially in intermediate-risk disease.

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Conflict of Interest

All authors have declared no competing interests.

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Abbreviations: AE, adverse event; Ara-C, cytosine arabinoside; CIS, carcinoma *in situ*; CTCAE, Common Terminology Criteria for Adverse Events; HR, hazard ratio; IRB, Institutional Review Board; MMC, mitomycin C; NMIBC, non-muscle-invasive bladder cancer; RFS, recurrence-free survival; TUR, transurethral resection; UTUC, upper tract urothelial cancer.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Survival curves of intent-to-treat population.

Table S1. Toxicities.