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Overactive bladder symptom severity is associated with falls in community-dwelling adults: LOHAS study

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ORIGINAL ARTICLE

Correlation between the Japanese Aging Male Questionnaire (JAMQ) and Aging Male's Symptom (AMS) scale in Japanese male

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Abstract

Objectives: To clarify the correlation between the Japanese Aging Male Questionnaire (JAMQ) and the Aging Males' Symptoms (AMS) scale through the factor analysis in Japanese male.
Materials and methods: In 61 male patients who visited the LOH outpatient clinic of Teikyo University Hospital, subjective symptoms featuring LOH were evaluated using the JAMQ and AMS. Factor analysis was performed on each questionnaire to clarify the LOH-related factors. Correlational analysis between the subscale scores representing such factors and the serum hormone profiles was also performed.
Results: Factor analysis of the JAMQ revealed an internal structure consisting of three subgroups: somatic, psychological and sexual factors with good categorization of the indicators to the appropriate subgroup. In contrast, the indicators of the AMS showed incomplete conformity to the subgroups of the JAMQ. Correlational analysis showed that each score on the JAMQ subgroups had the highest coefficient of correlation with the corresponding AMS subgroup ($p < 0.001$). There was no significant association between total and free serum testosterone levels and the total and subscale scores on either AMS or JAMQ.
Conclusions: The results of factor analysis suggest that the sexual perceptions of Japanese populations might differ from those of Caucasian populations. JAMQ would be useful to separately assess individual aspects of somatic, psychological and sexual symptoms related to LOH among Japanese males.

Keywords

Aging Males' Symptoms rating scale, Japanese Aging Male Questionnaire, late onset hypogonadism, testosterone replacement therapy

History

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Introduction

Testosterone and its metabolites play a crucial role in the health and development of males. Decreased testosterone levels cause a range of symptoms including sexual dysfunction, cognitive impairment, decreased energy, depressed mood, increased fat mass, sarcopenia, anemia and reduced bone mineral density [1]. Low testosterone levels can also have an impact on physical, social, emotional, cognitive and sexual functioning [2], all of which are key components of health-related quality of life (HRQOL) [3]. In the case of male hypogonadism, decreased energy levels and impaired sexual performance appear to be the most important QOL issues. The negative impact of hypogonadism on sexual function, energy level, body composition, mood and cognitive function is likely to have an adverse effect on QOL.

Late onset hypogonadism (LOH) is a clinical and biochemical syndrome associated with advancing age. It is characterized by the presence of any of the typical signs or symptoms of LOH listed above and a deficiency in serum testosterone levels [4–6]. Patients with LOH complaint about somatic, psychological and sexual symptoms such as increase in visceral fat, decrease in muscle volume and strength, change in mood, fatigue, depressed mood and decreased sexual functions. Many of these symptoms are similar to those associated with female menopause and depression [7]. LOH becomes a target of treatment when the clinical symptoms are related to a reduction in testosterone levels. Thus, the qualitative and quantitative evaluation of symptoms of LOH by screening questionnaires is essential to determining the severity of the syndrome. Furthermore, such questionnaires are useful for evaluating the efficacy of treatment.

The Aging Males' Symptoms (AMS) scale is a self-rating questionnaire that was originally developed and validated in a German database, that is, predominantly Caucasian. It consists of 17 items of psychological, somatic and sexual symptoms. It has been used worldwide to evaluate the severity of symptoms of LOH [8–10]. It also has been shown to be effective for evaluating the therapeutic efficacy of

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testosterone replacement therapy in the treatment of LOH [11]. It is the most frequently used tool for measuring HRQOL in aging males [12]. The scale was designed and standardized as a self-administered questionnaire to (1) assess symptoms of aging (independent from those which are disease-related) between groups of males under different conditions, (2) evaluate the severity of symptoms over time and (3) measure changes pre- and post-androgen replacement therapy.

The scale began as a listing of symptoms/complaints and a comparison of more than 200 variables in more than 100 medically well-characterized males. During recent years, international research has contributed to the development of the AMS scale as a patient-reported outcome scale used in clinical studies in all age groups of men. Furthermore, the AMS scale is well accepted internationally and is available in 21 languages [9]. Indeed, comparison of the internal structure of the German sample against samples from other countries shows a high degree of similarity, with a few notable exceptions. In the Asian samples, three out of five items intended to categorize in the psychological factor subgroup seem to associate more with the somatic subgroup. This finding suggests that the individual domain scores of the AMS are not fully independent in the Asian samples.

Kumamoto et al. conducted a community-based study ($n > 1000$) on quality of life (QOL) in healthy men and women between age 30 and 60, using a questionnaire that included items on mental and neurological symptoms, symptoms related to cardiovascular disorders, symptoms related to locomotive disorders, perceptual disorders and symptoms of sexual functions [11,13]. Using this questionnaire as a starting point, we developed the Japanese Aging Male Questionnaire (JAMQ) consisting of 18 questions (Table 1). The JAMQ was specifically designed to overcome the aberrant factorization of sexual perceptions among Japanese subjects taking the AMS. This scale has three dimensions of symptoms/complaints: psychological factors, somatic factors and sexual and urinary factors.

The purpose of this study was to assess the correlation between JAMQ and the AMS in Japanese male to demonstrate the usefulness of the JAMQ for clinical application. To complete our research task, factor analysis of the AMS and JAMQ was carried out individually to compare the effectiveness of each tool in assessing LOH. Our particular interest was to determine how clearly each questionnaire reflected the three subgroups of symptoms (psychological, somatic and sexual) identified by the factor analysis.

Materials and methods

Subjects

The subjects of this research were 61 patients who self-referred for symptoms of LOH either to the Men's Health Clinic of Teikyo University Hospital, Tokyo, or its related facilities. This study was reviewed by the Institutional Ethics Committee of Teikyo University, and all subjects gave their informed consent prior to being included in the study.

Methods

At the time of first visit, subjective symptoms were evaluated using the AMS and JAMQ questionnaires. Blood samples were collected between 9:00 and 11:00 am. Total and free serum testosterone levels were measured as part of the endocrinological examination.

Table 1 shows the items in JAMQ translated into English. (The English version of JAMQ has not yet been validated.) The JAMQ has 18 items and the first 17 are structured such that the individual subject can score the intensity of the symptoms on an ordinal scale of 1 to 4, that is, 1 stands for "almost none", 2 for "moderate", 3 for "severe" and 4 for "very severe". Only question 18 is differently structured to assess the frequency of sexual intercourse. Consequently, item

Table 1. JAMQ.

		None	Moderate	Severe	Very severe
1	Feel generally ill and tend to be in a bad mood	1	2	3	4
2	Have difficulty sleeping	1	2	3	4
3	Feel anxious/lonely	1	2	3	4
4	Tend to be more nervous and feel depressed	1	2	3	4
5	Experience hot flushes and excessive sweating	1	2	3	4
6	Experience palpitations, shortness of breath, and sometimes have difficulty breathing	1	2	3	4
7	Experience dizziness and nausea	1	2	3	4
8	Get tired easily	1	2	3	4
9	Have pain in my lower back and in the joints of my hands and feet	1	2	3	4
10	Have headaches, feel heavy-headed, and have stiff shoulders	1	2	3	4
11	Feel stiffness in my arms and legs	1	2	3	4
12	Feel numbness or tingling in my arms or feet	1	2	3	4
13	Find it hard to urinate or it takes a long time to finish urinating	1	2	3	4
14	Frequently feel the urge to urinate during the night.	1	2	3	4
15	Have difficulty controlling my bladder and sometimes experience incontinence	1	2	3	4
16	Feel that my libido has decreased	1	2	3	4
17	Have diminished erectile strength	1	2	3	4
18	Frequency of sex	>1, 2 times in 2 weeks	1, 2 times/month	<once/month	None

18 was excluded from the analysis when calculating the total scores [9]. Table 2 shows the items in the AMS. Each item has five degrees of severity of symptoms [8–10].

Data analysis

The analyses were performed using the SAS statistical package (SAS Institute Inc., Cary, NC). A p value of less than 0.05, two-tailed, was considered statistically significant. First, a factor analysis was conducted to independently determine the structure of AMS items versus the JAMQ items. Common factors were extracted from the items by factor analysis (principal component analysis) provided that each eigenvalue was greater than 1.0. Eigenvalues measure the amount of variation in the total sample accounted for by each factor. If a factor has a low eigenvalue, then it is contributing little to the explanation of variances in the variables and may be disregarded as being redundant with more important factors. The common factor axes were rotated by the varimax method to show the distinct nature of each factor. These methods are standardized and commonly used in studies involving factor analysis [14,15]. Second, correlational analysis was performed between the individual item scores of each factor of AMS and JAMQ, respectively. Finally, correlational analysis was also performed between the scores obtained on the AMS and JAMQ and serum levels of total and free testosterone.

Results

Patients' characteristics

Table 3 shows the patients' characteristics. Thirty-two subjects were under 50, 24 subjects were between 50 and 64, and 4 subjects were older than 65 years of age. The median values of total and free serum testosterone were 367.7 ng/dl and 12.1 pg/ml, respectively; no significant differences in these levels were found among the different age groups. The median scores of total JAMQ were 35, whereas those of the total AMS score were 46. No significant differences in scoring were found between the different age groups.

Factor analysis of the questionnaires

Five factors had eigenvalues of more than 1 in both the AMS and the JAMQ. Concerning AMS, the eigenvalues for the first through fifth factors were 7.46, 2.12, 1.32, 1.12 and 1.08, respectively. Concerning JAMQ, they were 6.73, 1.76, 1.35, 1.17 and 1.07, respectively. Factor analysis was then performed setting the significant number of factors to 3, because AMS is usually divided into three categories, namely, somatic, psychological and sexual, and because the effectiveness of doing so was confirmed in a previous study [16]. Figure 1 shows the results of this analysis. The cumulative proportion rates of the three factors were 77% for AMS and 58% for JAMQ. The numbers shown are the highest factor loadings for each of the questions. In the original AMS, questions 1 through 5, and 9 through 10 were classified as somatic factors, but questions 2 and 3 were classified separately into Factor 3 in this study. The questions originally categorized as psychological factors, questions 6 through 8, 11 and 13 were all classified into Factor 1 together with the somatic factors. The questions originally categorized as sexual factors, questions 12 and 14 through 17 were partly classified into Factor 2 (questions 15 through 17) and partly into Factor 3 (questions 12 and 14) in this study.

When AMS was applied to the subjects in the present study, the three subgroups proposed by Heinemann [8] did not fit well. On the other hand, in JAMQ, when Factor 1 was considered psychological, Factor 2 as sexual, and Factor 3 as somatic, it matched very well with the content of the questions. However, question 10 "Have headaches, feel heavy-headed and suffer from stiff shoulders" was classified as Factor 1, although the question was originally intended to reflect somatic symptoms. The reason for this discrepancy is addressed in the discussion, and we finally divided JAMQ into the three subgroups as shown in Table 4.

Correlational analysis of the questionnaires

Table 5 shows the results of the correlational analyses between the AMS and JAMQ scores. Each score on the three factor items had the highest coefficient of correlation with that of corresponding factors in the other scale ($p<0.001$). The correlation analysis showed the significant variation in the coefficient of correlation between AMS and JAMQ. For example, sexual domain in AMS had the highest coefficient with psychological domain in JAMQ. Table 6 shows the results of correlation analysis between biological parameters (i.e. total and free serum testosterone levels) and the response to questionnaires (i.e. the AMS and JAMQ scores). There were no significant associations between the biological parameters and the total scores of AMS and JAMQ or with the scores of each factor of AMS and JAMQ.

Discussion

This study supports the prevalent 3-factor model of LOH, namely, somatic, psychological and sexual [7,17–23]. In this regard, JAMQ conformed better with the pre-existing model than the AMS in Japanese subjects. The AMS was presented for the first time in 1999 by Heinemann [8]. Subsequently, this questionnaire was translated into various languages, including Japanese, and its validity was tested in clinical subsets from different countries [9]. To date, there have been a few reports of problems with the Japanese version of AMS [24–26]. Indeed, five of the seven somatic items seemed not to be associated with the somatic factor [26]. The attitudes toward sex in the Japanese culture differ from those of other cultures; we have had concerns about the validity of using the AMS in Japanese subjects. For example, AMS question 12 reads: "Feeling that you have passed the zenith of life". Unlike questions 15 through 17, one would not expect a Japanese male to interpret question 12 as being related to sexual symptoms. In Japan the phrase "apex or zenith of one's life" evokes psychological or somatic factors; and most Japanese men will tend to associate this indicator with work, family, and physical potential. Therefore, Japanese subjects would be more likely to interpret question 12 as a request for information about one's outlook on life as it relates to somatic or psychological symptoms as opposed to a sexual factor. Indeed, in this analysis, this question was categorized as

Table 2. AMS Questionnaire.

Which of the following symptoms apply to you at this time? Please, mark the appropriate box for each symptom. For symptoms that do not apply, please mark "none".

	Symptoms:	none	mild	moderate	severe	Extremely severe
	Score=	1	2	3	4	5
1.	**Decline in your feeling of general well-being** (general state of health, subjective feeling)	☐	☐	☐	☐	☐
2.	**Joint pain and muscular ache** (lower back pain, joint pain, pain in a limb, general back ache)	☐	☐	☐	☐	☐
3.	**Excessive sweating** (unexpected/ sudden episodes of sweating, hot flushes independent of stain)	☐	☐	☐	☐	☐
4.	**Sleep problems** (difficulty in falling asleep, difficulty in sleeping through, waking up early and feeling tired, poor sleep, sleeplessness)	☐	☐	☐	☐	☐
5.	**Increased need for sleep, often feeling tired**	☐	☐	☐	☐	☐
6.	**Irritability** (feeling aggressive, easily upset about little things, moody)	☐	☐	☐	☐	☐
7.	**Nervousness** (inner tension, restlessness, feeling fidgety)	☐	☐	☐	☐	☐
8.	**Anxiety** (feeling panicky)	☐	☐	☐	☐	☐
9.	**Physical exhaustion/lacking vitality** (general decrease in performance, reduced activity, lacking interest in leisure activities, feeling of getting less done, of achieving less, of having to force oneself to undertake activities)	☐	☐	☐	☐	☐
10.	**Decrease in muscular strength** (feeling of weakness)	☐	☐	☐	☐	☐
11.	**Depressive mood** (feeling down, sad, on the verge of tears, lack of drive, mood swings, feeling nothing is of any use)	☐	☐	☐	☐	☐
12.	**Feeling that you have passed your peak**	☐	☐	☐	☐	☐
13.	**Feeling burnt out, having hit rock-bottom**	☐	☐	☐	☐	☐
14.	**Decrease in beard growth**	☐	☐	☐	☐	☐
15.	**Decrease in ability/frequency to perform sexually**	☐	☐	☐	☐	☐
16.	**Decrease in the number of morning erections**	☐	☐	☐	☐	☐
17.	**Decrease in sexual desire/libido** (lacking desire for sexual intercourse)	☐	☐	☐	☐	☐
	Have you got any other major symptoms?	Yes	☐	No	☐	
	If Yes, please describe:					

Factor 3 (somatic) together with question 2 "Complaints in joints or muscles" and question 3 "Sweating". It is difficult to find adequate words in English to describe the images these questions evoke. The most we can say is that question 12 is definitely different from questions 15–17 that ask directly about sexual conditions. When complementarity with AMS is considered, JAMQ might be more useful if it is evaluated by dividing it into three factors. Future studies will have to determine the validity of JAMQ in evaluating the efficacy of therapeutic treatments for LOH.

Question 10 of the JAMQ (i.e. headache) was also misclassified as Factor 1 (psychological) in the factor analysis of this study. We offer the following interpretation of this apparently inconsistent result. About 50% of patients who visit the Men's Health clinic in Japan have a history of visiting neuropsychiatrists or psychosomatic (i.e. alternative) internal medicine practitioners for depression [11]. Thus, they principally complain of psychological symptoms, such as depression, or vegetative symptoms, such as coldness in one's hands and feet or sweating. It is common in the Japanese culture for a psychological state to manifest as a somatic symptom. For example, Japanese people tend to "wrap one's arms over one's head" when they feel depressed. Hence, it is not unreasonable that symptoms of a headache would be classified as a psychological factor.

Uniquely, questions 13 through 15 in JAMQ ask about lower urinary tract symptoms (LUTS). (13, Find it hard to urinate or it takes a long time to finish urinating. 14, Frequently feel the urge to urinate during the night. 15, Have difficulty controlling my bladder and sometimes experience incontinence.) AMS does not contain the question about LUTS; however, JAMQ was designed to assess LUTS to

Table 3. Patients' characteristics.

	Age group (years)			Total
	<50	50–64	≥65	
Number of patients	32	24	4	61
				36–71
Testosterone levels	349.3	368.7	331.1	367.7
(ng/dl)	54.5–760.8	72.6–810.8	228.2–603.2	54.4–810.8
Free testosterone	11.5	14.1	9.7	12.1
levels (pg/ml)	2.4–19	2.2–22.7	6.3–13.8	2.2–27.5
Total JAMQ	35.5	34	36	35
Scores (Q1–17)	18–62	20–34	29–42	18–62
Total AMS Scores	46.5	43	42.5	46
	23–73	22–69	40–47	22–73

Upper row: Median, Lower row: Minimum - Maximum values
Total testosterone levels, free testosterone levels, total JAMQ scores and total AMS scores were not significantly different between all age groups.

Table 4. Subgroups of JAMQ.

Subgroups	Questions	# of questions	Scoring
Somatic factors	6, 7, 9, 10, 11, 12	6	24 total points
Psychological factors	1, 2, 3, 4, 8	5	20 total points
Sexual factors	5, 13, 14, 15, 16, 17	6	24 total points
Sexual activity	18	1	4-point scale

Because sexual activity is asked in a different way, this question is not included with the other questions for statistical analysis. Sexual activity was analyzed as a separate factor additionally because it is believed that standard sexual activity varies greatly by individual.

Table 5. Coefficient of correlation between the JAMQ and AMS scores.

	AMS			
	Total	Somatic factors	Psychological factors	Sexual factors
JAMQ				
Total	0.844	0.856	0.851	0.884
Somatic factors	0.635	0.673	0.641	0.635
Psychological factors	0.856	0.838	0.912	0.856
Sexual factors	0.670	0.613	0.591	0.670

$p < 0.0001$.
The totals of the AMS and JAMQ scores and the factors that were common in both tests had a coefficient of correlation between 0.670 to 0.912 and showed significant correlation.

Figure 1. Factor Patterns of AMS and JAMQ (Varimax Rotation).

	AMS		
	Factor 1	Factor 2	Factor 3
1	0.77968		
2			0.51119
3			0.47143
4	0.71089		
5	0.65927		
6	0.79815		
7	0.86634		
8	0.86332		
9	0.80179		
10	0.53589		
11	0.87268		
12			0.80080
13	0.61007		
14			0.29105
15		0.83390	
16		0.86225	
17		0.73909	

	JAMQ		
	Factor 1	Factor 2	Factor 3
1	0.75167		
2	0.61789		
3	0.77095		
4	0.83799		
5		0.30906	
6			0.93924
7			0.59886
8	0.62110		
9			0.54614
10	0.77482		
11			0.85578
12			0.73413
13		0.65150	
14		0.61214	
15		0.57032	
16		0.63548	
17		0.85333	

Somatic factors | Psychological factors | Sexual factors

Table 6. Correlation of JAMQ and AMS scores with the levels of sexual hormones.

	JAMQ			AMS		
	Somatic	Psychological	Sexual	Somatic	Psychological	Sexual
Testosterone levels	−0.010	0.048	0.167	−0.064	0.052	0.104
	0.9376	0.7173	0.2025	0.6276	0.6968	0.4297
Free testosterone levels	0.094	0.154	0.121	0.001	0.144	0.162
	0.4912	0.2598	0.889	0.9963	0.2921	0.2333

Upper row: Coefficient of correlation, Lower row: *p*
Each subgroup of the AMS and JAMQ scales did not correlate with the testosterone and free testosterone levels.

enable us to obtain the information regarding LUTS induced via aging. In the current study, we analyzed JAMQ to categorize every question into three specific domains, such as somatic, psychogenic and sexual domain as AMS does. As results, all of these three questions in JAMQ were categorized in the sexual domain. Community-based and clinical data demonstrate a strong and consistent association between LUTS and ED, suggesting that elderly men with LUTS should be evaluated for sexual dysfunction and vice versa [27]. Our study confirmed that questionnaires on LUTS were associated with sexual function by factor analysis. JAMQ is a unique questionnaire that can assess LUTS in LOH patients.

We should address some limitations in this study. First, we examined the correlation between AMS and JAMQ in the heterogeneous population, in which there are male subjects whose testosterone are normal and the symptoms are mild. This study conceives a nature of the pilot study for the clinical application among LOH patients in Japan. Therefore, in this study, total scores of both AMS and JAMQ were not significantly associated with total and free serum testosterone. Thus, neither the AMS nor JAMQ might be suitable tools for screening of LOH. However, recent study shows the testosterone changes in LOH patients are more important and predictive for the LOH symptoms [28]. We need to assess the correlation between the testosterone change and both questionnaires score in the future. Moreover, we should assess the LOH patients with low testosterone level to confirm the exact potential as the screening tool of JAMQ.

This study supports several important conclusions. First, one cannot assume the validity of a questionnaire in other cultures. Factor analysis of the internationally accepted AMS and the JAMQ demonstrated better conformity of the JAMQ to the pre-existing 3-factor model of LOH and less than optimal factorization of the AMS in items pertaining to sexual perceptions in the Japanese population. Whether the differences are semantic or cultural, any questionnaire that has been translated into another language should be subject to rigorous factor analysis before it is applied to the sample. The second and more surprising conclusion is that, cultural differences aside, neither the AMS nor the JAMQ correlated with the signs and symptoms of LOH. This finding seriously limits the relevance of both questionnaires as a screening tool for the identification of LOH, and definitive diagnosis on a clinical basis alone remains out of reach. The JAMQ may still be useful to separately assess the individual aspects of somatic, psychological and sexual symptoms related to LOH in Japanese populations. It remains to be seen whether the JAMQ will be effective for evaluating the therapeutic efficacy of testosterone replacement therapy in the treatment of LOH, and the JAMQ, itself, requires future validation in English.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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Maintenance Therapy with Intravesical Bacillus Calmette–Guérin in Patients with Intermediate- or High-risk Non-muscle-invasive Bladder Cancer

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Objective: We investigated the efficacy, safety and an optimal schedule of maintenance therapy with intravesical instillation of Bacillus–Calmette Guérin in patients with non-muscle-invasive bladder cancer.

Methods: We compared the oncological outcome and adverse events of maintenance Bacillus–Calmette Guérin therapy ($n = 40$) with control subjects ($n = 64$) of Bacillus–Calmette Guérin induction therapy. Maintenance therapy was scheduled to be administered in 3-week cycles at 6, 12, 18, 24 and 36 months after the induction therapy.

Results: There was a significant difference in the 5-year recurrence-free survival rate between the maintenance and induction groups in all patients (72.4 vs. 62.0%; $P = 0.019$) and in patients with high recurrence risk (100.0 vs. 17.9%; $P = 0.009$). There was a significant difference in the 5-year progression-free survival rate between the maintenance and induction groups in patients with high progression risk (100.0 vs. 69.3%; $P = 0.047$). Maintenance Bacillus–Calmette Guérin instillations for a total of four times or more (recurrence-free survival: hazard ratio: 0.2, $P = 0.039$) or with a total dosage of >243 mg (recurrence-free survival: hazard ratio: 0.2, $P = 0.041$) after 6 months of induction therapy significantly improve tumor recurrence-free survival and progression-free survival. There were no significant differences between induction therapy and maintenance therapy in the frequency of all adverse drug reactions.

Conclusions: Bacillus–Calmette Guérin maintenance therapy was effective in preventing the recurrence and progression of high-risk non-muscle-invasive bladder cancer. Maintenance Bacillus–Calmette Guérin instillations for a total of four times or more or with a total dosage of >243 mg after 6 months of induction therapy are necessary to obtain the optimal effect as maintenance therapy.

Key words: BCG – non-muscle-invasive bladder cancer – maintenance therapy

INTRODUCTION

Non-muscle-invasive bladder cancer (NMIBC) recurs in up to 80% of cases and progresses in up to 55% (1). To date, there have been numerous reports concerning the use of prophylactic intravesical instillation therapy after transurethral resection of bladder cancer (TUR-Bt). As a result, many guidelines currently recommend a standard treatment of TUR-Bt with a curative intent, followed by adjuvant intravesical instillation.

In intermediate- or high-risk patients, intravesical instillation of Bacillus–Calmette Guérin (BCG) as induction and maintenance therapies is recommended (2). Several large studies have reported the efficacy of BCG maintenance therapy. A meta-analysis showed that only BCG instillation as a maintenance therapy prevented the progression of NMIBC (3).

The European Association of Urology guidelines on TaT1 bladder cancer strongly recommended that BCG must be given in a maintenance schedule (level of evidence: 1a) (4) on the basis of a reduction of 37% in the odds of progression ($P = 0.00004$) in 20 trials in which some form of BCG maintenance was given (5). The American Urological Association's Guidelines for the Management of NMIBC provide only a moderate recommendation for BCG maintenance therapy in patients with high-risk NMIBC (6). Likewise, the National Comprehensive Cancer Network guidelines (V.I.2010) state that maintenance therapy with BCG is optional in this setting (7). Thus, although BCG maintenance therapy is recommended to prevent the recurrence or progression in patients with high-risk NMIBC, the recommendation differs somewhat from guideline to guideline.

Many different maintenance schedules have been used, but the optimal schedule of induction and maintenance instillations remain unknown. One meta-analysis was unable to determine which BCG maintenance schedule was the most effective (5). In the Southwest Oncology Group (SWOG) famous randomized study (8), the maintenance period was longer than 2 years, and a total of 21 maintenance instillations were administered. The administration of multiple instillations, however, leads to higher morbidity and the subsequent discontinuation of treatment. Consequently, in this study, only a small percentage of patients were able to complete the 3-year maintenance dosing schedule. Nevertheless, the 3-year maintenance BCG therapy has been performed empirically in a similar way until now at many institutions without therapeutic rationale. Therefore, we must establish the adequate BCG maintenance schedule to enhance the most highly efficacy in cancer control and produce the fewest side effects as soon as possible.

In this study, we retrospectively evaluated the efficacy and safety of intravesical BCG maintenance immunotherapy in preventing recurrence and progression of NMIBC with the aim of developing an optimal maintenance schedule.

PATIENTS AND METHODS

Patients with histologically confirmed Ta, T1 urothelial carcinoma or carcinoma *in situ* (CIS) of the bladder were eligible for inclusion in the study. The TNM classification was assessed according to the general rules for clinical and pathological studies on renal pelvic, ureteral and bladder cancers as detailed in the Japanese Urological Association's 2011 classifications (9). Risk group classification was performed in accordance with the guidelines of the European Association of Urology (1). Patients with any of the following were excluded from the analysis: (i) history of muscle-invasive or metastatic bladder cancer; (ii) history of carcinoma of the urethra, prostate (ducts or stroma) or upper urinary tract; (iii) history of local radiation therapy to the pelvis; (iv) history of intra-arterial or systemic chemotherapy; (v) history of previous BCG therapy; (vi) classification as low risk for recurrence and progression according to the European Association of Urology guideline (1); (vii) hydronephrosis; (viii) strongly positive tuberculin reaction or active tuberculous lesion; (ix) residual urine of >150 ml after urination and severe bladder irritation before the start of drug administration; (x) active double cancer or other serious medical complications.

Patients with previous intravesical instillation of chemotherapeutic agents were eligible for inclusion provided they had discontinued treatment at least 6 months prior to the start of BCG induction therapy. At least 6 weeks after TUR-Bt, patients received the initial 4–8 weekly intravesical administrations of 81 mg of BCG (Connaught strain) in 40 ml of physiological bacteriostatic preservative-free saline solution per each week. In the case of moderate-to-severe adverse drug reaction (ADR), dosage reduction to 27 mg of Connaught strain and delay in administration not for longer than 3 weeks from the scheduled administration day was allowed. The solution was instilled into an emptied bladder through a catheter. All patients were instructed and confirmed to retain the solution for 2 h after instillation, changing position every 15 min.

After 2006, we recommended the maintenance therapy for all patients after induction therapy. Maintenance therapy was administered weekly for 3 weeks, starting at 6, 12, 18, 24 and 36 months after TUR-Bt. For maintenance therapy as well as induction therapy, in the event of ADRs, dosage reduction to 27 mg and delay in administration was allowed. Patients given even one instillation as maintenance therapy were included in the maintenance group. We included the patients with only induction therapy before 2006 as control subjects. To prevent severe ADR, we administrated antibiotics (new quinolones) to all patients for 3 days not only during induction therapy but also during maintenance therapy.

We retrospectively compared the oncological outcomes between the maintenance and induction groups. Patients in both the groups underwent urinalysis, urinary cytology and cystoscopy after BCG instillation. These examinations were repeated every 3 months for the first 3 years and thereafter every 6 months in order to observe the presence or absence of recurrence and progression. Visible recurrences or suspicious lesions were removed by TUR-Bt and biopsy. Whenever disease progression was suspected, imaging tests such as computed tomography scans were performed. All recurrences were confirmed by histopathology, and progression was defined as muscle-invasive tumor or metastatic disease. Progression was also regarded as recurrence.

Table 1. Baseline characteristics of patients

	Total	Induction group	Maintenance group	*P* value
n	104	64	40	
Male (%)	90 (85.7)	54 (84.4)	36 (90.0)	0.414
Median age in years (range)	68 (44–84)	68.5 (50–84)	65.5 (44–81)	0.271
Tumor status (%)				
Primary	56 (53.8)	34 (53.1)	22 (55.0)	0.852
Recurrent	48 (46.2)	30 (46.9)	18 (45.0)	
pT classification				
pTis	18 (17.3)	13 (20.3)	5 (12.5)	0.505
pTa	57 (54.8)	35 (54.7)	22 (55.0)	
pT1	29 (27.9)	16 (25.0)	13 (32.5)	
Grade				
Low	58 (55.8)	31 (48.4)	27 (67.5)	0.057
High	46 (44.2)	33 (51.6)	13 (32.5)	
Tumor size >3 cm (%)	12 (11.5)	7 (10.9)	5 (12.5)	0.808
Multiplicity (%)	69 (66.3)	41 (64.1)	28 (70.0)	0.533
Concomitant CIS (%)	15 (14.4)	10 (15.6)	5 (12.5)	0.659
Recurrence risk				
Intermediate	85 (81.7)	51 (79.7)	34 (85.0)	0.495
High	19 (18.3)	13 (20.3)	6 (15.0)	
Progression risk				
Intermediate	52 (50.0)	28 (43.8)	24 (60.0)	0.107
High	52 (50.0)	36 (56.3)	16 (40.0)	
2nd TUR-Bt (%)	28 (26.9)	18 (28.1)	10 (25.0)	0.727
Previous use of chemotherapeutic agents	25 (24.0)	13 (20.3)	12 (30.0)	0.261

Recurrence-free survival (RFS) and progression-free survival (PFS) were defined as the period elapsed between the last BCG induction instillation and recurrence or progression, respectively.

The significance of differences in the patient background factors between the two groups was examined by the χ^2 and *t*-test. RFS and PFS curves were calculated by the Kaplan–Meier method, and statistical significance between the groups was determined by the log-rank test. The significance of differences in the total number or the total dosage of BCG instillations between patients with and without recurrence or progression was examined by the *t*-test. Univariate and multivariate Cox proportional hazard models were used to estimate the prognostic factor on RFS and PFS.

ADRs were divided into local (bladder-related) side effects and systemic side effects. Both local and systemic ADRs were monitored at every visit and assessed according to the Common Terminology Criteria of Adverse Events version 4.0. During induction or maintenance periods, local and systemic ADRs were graded and recorded. Statistical analysis was done using R (version 2.14.0). Statistical significance was determined at $P < 0.05$. All tests were two-sided.

RESULTS

A total of 104 patients were enrolled at our institute in this retrospective study. The tumor characteristics of the two groups were well balanced (Table 1). There were no significant differences in the follow-up period between the two groups (induction group: 42.3 ± 33.1 month, maintenance group: 51.1 ± 34.6 month, $P = 0.207$). Histological typing showed all patients except one in the maintenance group and two in induction group had urothelial cancer. In patients in the maintenance group, the median number of maintenance BCG instillations was four times and the median dose of maintenance BCG instillation was 243 mg.

Of the 40 patients in the maintenance group, 12 (30.0%) completed the planned 3-year maintenance treatment. The highest percentage of all the three instillations per cycle was observed at the 12th month (61.9%) compared with other cycles (6th month: 25.2%, 18th month: 31.6%, 24th month: 41.9%, 36th month: 26.3%). During follow-up periods, 29 patients (27.9%) experienced recurrence and 8 (7.7%) progressed to muscle-invasive disease or metastasis. There was no significant difference in the total number and total

Table 2. The total number and total dosage of induction and/or maintenance BCG therapy

	Recurrence			Progression		
	+	−	*P* value	+	−	*P* value
n	28	76		7	97	
Total number of induction BCG instillations	6.8 ± 1.4	6.8 ± 1.6	0.918	7.3 ± 1.4	6.8 ± 1.6	
Total dosage of induction BCG instillations (mg)	456.7 ± 189.0	481.6 ± 185.9	0.542	469.1 ± 190.9	475.1 ± 186.8	0.934
Period of delay in scheduled instillation of induction BCG (weeks)	1.2 ± 2.8	2.2 ± 3.4	0.127	—	—	—
Total number of induction + maintenance BCG instillations	7.7 ± 2.4	8.9 ± 4.1	0.070	7.6 ± 1.7	8.7 ± 3.8	0.168
Total dosage of induction + maintenance BCG instillations (mg)	520.9 ± 265.1	632.2 ± 368.7	0.091	499.5 ± 225.0	609.9 ± 353.3	0.234
Total number of maintenance BCG instillations	0.9 ± 2.0	2.1 ± 3.2	0.021	0.4 ± 1.1	1.9 ± 3.0	0.007
Total dosage of maintenance BCG instillations (mg)	64.2 ± 156.7	150.6 ± 250.0	0.038	30.4 ± 85.9	134.7 ± 237.1	0.015

The differences in the total number, total dosage and the period of delay in scheduled instillation of induction with or without maintenance BCG therapy did not influence either recurrence or progression. There was, however, significant difference in the total number and total dosage of maintenance BCG instillation between patients with and without recurrence or progression.

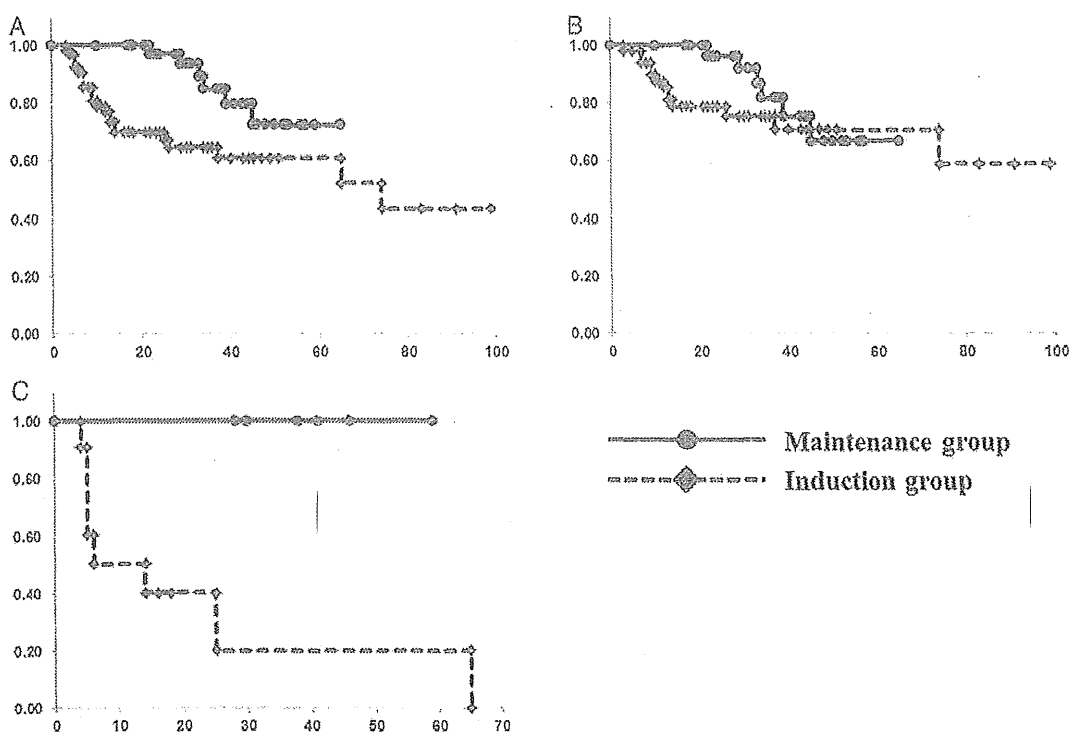

Figure 1. (A) Kaplan–Meier 5-year RFS analysis shows that patients who received at least one maintenance BCG instillation had a significantly reduced risk of recurrence compared with patients who received induction BCG only ($P = 0.019$). (B) Comparison of patients with intermediate recurrence risk bladder cancer who received at least one cycle of maintenance BCG instillations and patients with an intermediate recurrence risk who received induction BCG only ($P = 0.389$). (C) Patients with high recurrence risk bladder cancer who received at least one cycle of maintenance BCG instillation had a significantly reduced risk of recurrence compared with patients who received induction BCG only. ($P = 0.009$).

dosage of induction or induction + maintenance BCG instillation between patients with and without recurrence or progression (Table 2). Moreover, there was no significant difference in the weeks of delay in instillation of induction BCG between patients with and without recurrence or progression (Table 2). Thus, differences in the total number, total dosage and period of delay to induction of BCG instillation did not influence either recurrence or progression in this study.

Recurrence was confirmed in 6 patients (15.0%) in the maintenance group and in 23 (35.9%) in the induction group. The estimated Kaplan–Meier 5-year RFS rate was

Table 3. Univariate and multivariate Cox proportional hazard analysis of recurrence factors

Variable	Univariate			Multivariate		
	Hazard ratio	95% CI	*P* value	Hazard ratio	95% CI	*P* value
Age						
<68	1	0.7–3.4	0.230	1	0.7–3.7	0.265
≥68	1.6			1.6		
Sex						
F	1	0.2–1.7	0.369	1	0.2–1.8	0.443
M	1.6			1.6		
pT						
pTa	1	0.5–2.9	0.697	1	0.3–2.2	0.730
pT1	1.2			1.2		
CIS						
No	1	0.7–3.3	0.248	1	0.2–1.3	0.155
Yes	1.6			1.5		
Grade						
Low	1	2.0–12.4	0.001	1	1.8–15.5	0.002
High	5.0			5.3		
Disease						
No prior	1	0.4–1.8	0.709	1	0.7–3.8	0.244
Recurrent	0.9			1.6		
Prior intravesical chemotherapy						
No	1	0.1–0.7	0.013	1	0.0–1.0	0.048
Yes	0.2			0.2		
Induction BCG						
<6	1	0.5–4.4	0.459	1	0.7–6.3	0.201
≥6	1.5			4.5		
Maintenance BCG						
No	1	0.1–0.8	0.016	1	0.1–0.9	0.023
Yes	0.3			0.2		

CI, confidence interval.

72.4% for patients in the maintenance group and 62.0% for those in the induction group. There was a significant difference in the 5-year RFS rate between the groups [hazard ratio (HR) = 0.341, $P = 0.019$, Fig. 1A]. Multivariate analysis revealed that the rate of recurrence was statistically associated with tumor grade (HR = 5.3), prior intravesical chemotherapy (HR = 0.2) and maintenance BCG instillation (HR = 0.2) (Table 3). In patients with intermediate-recurrence-risk NMIBC, there was no significant difference in the 5-year RFS rate between the groups ($P = 0.389$, Fig. 1B). In patients with high recurrence risk NMIBC, however, patients in the maintenance group had a significantly reduced risk of recurrence when compared with those in the induction group ($P = 0.009$, Fig. 1C).

Disease progression was seen in only one patient (2.5%) in the maintenance group and in seven patients (10.9%) in the induction group. The estimated Kaplan–Meier 5-year PFS rate was 96.3% for patients in the maintenance group and 84.8% for those in the induction group. There was no significant difference in the -year PFS rate between the groups ($P = 0.115$, Fig. 2A). Although univariate analysis revealed that the rate of progression was statistically associated with CIS (HR = 5.5), multivariate analysis did not show any clinical significant prognostic factor (data not shown). Although in patients with intermediate progression risk NMIBC there was no significant difference in the 5-year PFS rate between the maintenance and induction groups ($P = 0.364$, Fig. 2B), in patients with high progression risk NMIBC the 5-year PFS rate with the log-rank test indicates a significantly lower progression rate in the maintenance group than in the induction group ($P = 0.047$, Fig. 2C).

In this study, the maintenance schedule comprised 3 weekly instillations at 3, 6, 12, 18, 24 and 36 months. However, since the optimal dose and timing of maintenance instillations are unknown, it is not clear whether our maintenance schedule is appropriate. At first, we examined the time at which instillation is most critical to prevent tumor recurrence and progression. Because of the small sample size, we could not evaluate the effect of a 36-month instillation for maintenance therapy. We therefore investigated the efficacy of 6-, 12-, 18- and 24-month instillations for maintenance therapy. Univariate Cox proportional hazard models demonstrated that the respective institution at 6, 12, 18 and 24 months as maintenance therapy are not significant prognostic factors for recurrence, individually (Table 4). There was, however, significant difference in the total number and total dosage of maintenance BCG instillation between patients with and without recurrence or progression (Table 2). So, we examined the optimal total number and the total dosage of maintenance BCG instillations (Table 5). Compared with less than four times, maintenance BCG instillation of four times or more significantly improve the tumor recurrence (HR 0.2, $P = 0.039$, Table 5). As compared with 9.1% of progression rates in patients with maintenance BCG therapy less than four times, no cases with maintenance BCG therapy of four times or more show progression (Table 5). Similarly, in terms of the total dosage of maintenance BCG instillations, compared with 243 mg or less, maintenance BCG instillations with a total dosage of >243 mg significantly improve the tumor recurrence (HR: 0.2, $P = 0.041$, Table 5). As compared with 9.2% of progression rates in patients with a total dosage of 243 mg or less as maintenance BCG therapy, no cases with a total dosage of >243 mg as maintenance BCG therapy show progression (Table 5).

Although urination-related local ADRs occurred in 84.4% of patients in the induction group during the induction instillation, the frequency of Grade 2 urinary events was low, and there were no Grade 3 events. Table 6 shows the local and systemic ADRs observed during both the induction therapy and maintenance therapy in the maintenance group. There

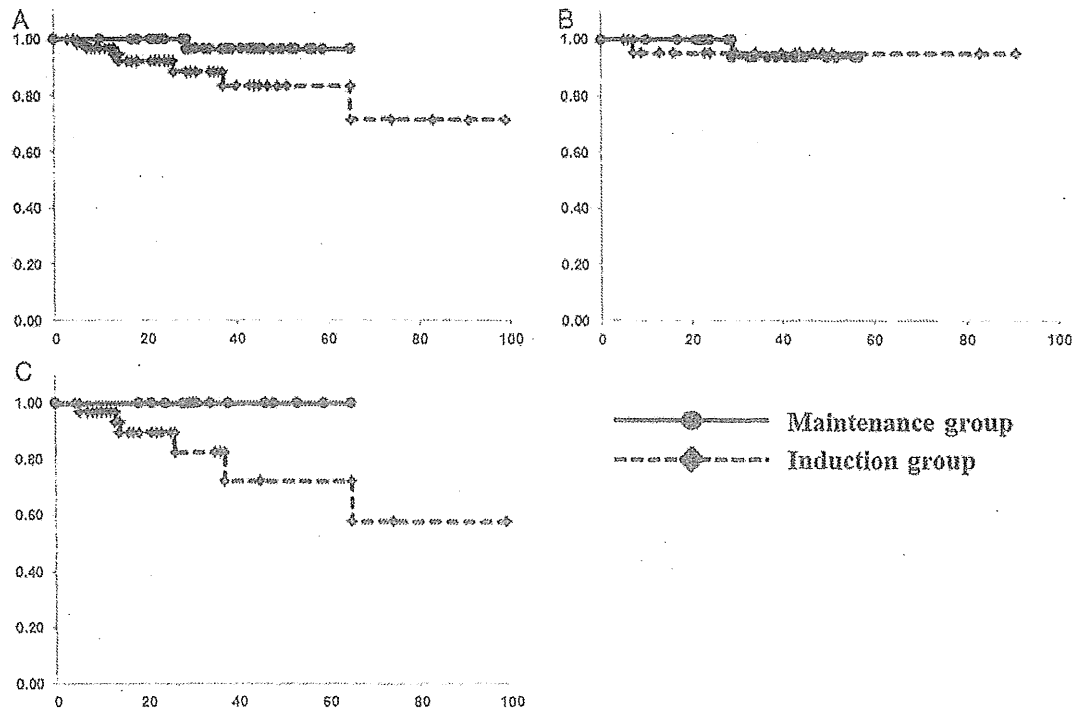

Figure 2. (A) The estimated Kaplan–Meier 5-year PFS rate was 96.3% for patients who received at least one cycle of maintenance BCG instillations and 84.8% for those who received induction BCG only. There was no significant difference between these groups ($P = 0.115$). (B) In the Kaplan–Meier PFS rate, there was no significant difference between patients with intermediate progression risk NMIBC in the maintenance and induction groups ($P = 0.364$). (C) In the Kaplan–Meier PFS rate, there was a significant difference between patients with high-progression-risk NMIBC in the maintenance and induction groups ($P = 0.047$).

Table 4. Univariate Cox proportional hazard models demonstrated that the respective institutions at 6, 12, 18 and 24 months as maintenance therapy are not significant prognostic factors for recurrence, individually

	RFS	Univariate Cox proportional hazard analysis		
		Hazard ratio	95% CI	*P* value
6 months	1/13 (7.7%)	0.2	0.0–1.6	0.128
12 months	6/27 (22.2%)	0.6	0.3–1.6	0.334
18 months	1/13 (7.7%)	0.2	0.0–1.5	0.122
24 months	1/13 (7.7%)	0.2	0.0–1.3	0.088

was no significant difference between the induction therapy and the maintenance therapy in the frequency of all local (induction: 57.5%, maintenance 67.5%, $P = 0.657$) and systemic (induction: 27.5%, maintenance 22.5%, $P = 0.606$) ADRs.

DISCUSSION

Intravesical BCG maintenance therapy has been often indicated as an adjuvant treatment following induction therapy to reduce the rate of recurrence and progression in patients with intermediate- or high-risk NMIBC. At present, intravesical BCG maintenance therapy is expected to play an established role in not only complementary therapy but also in its continuing effectiveness without local and systemic ADR. As a result, many guidelines worldwide recommend intravesical BCG maintenance therapy for patients with high-risk NMIBC (4,6,7,10). In this study, as with previous studies (3–5,8), patients with high-risk NMIBC who received maintenance BCG instillations had a significantly reduced risk of recurrence and progression compared with patients who received induction BCG only.

Although intravesical BCG instillation is often associated with undesirable ADRs, in the European Organization for Research and Treatment of Cancer prospective study, the majority of cases of discontinuation as a result of ADRs were seen during induction therapy and the first 6 months of maintenance therapy, suggesting that BCG maintenance does not necessarily increase the occurrence of adverse events (11). Another report concluded that there were fewer ADRs with maintenance therapy than with induction therapy (12). In line with these previous studies, there were also no significant differences in the frequency of all local and systemic ADRs between the induction therapy and maintenance therapy in the present study. Understandably, patients who tolerated well to BCG treatment during the induction therapy can receive maintenance therapy safely and comfortably.

Table 5. The maintenance BCG instillations for a total of four times or more or with a total dosage of >243 mg after 6 months of induction therapy significantly improve tumor RFS and PFS

	RFS				PFS			
	n	Hazard ratio	95% CI	*P* value	*n*	Hazard ratio	95% CI	*P* value
Total number of maintenance BCG instillations								
<4	27/83 (32.5%)	1	0.1–0.9	0.039	8/83 (9.1%)	–	–	–
≥4	2/21 (9.5%)	0.2			0/21 (0%)	–		
Total dosage of maintenance BCG instillations (mg)								
≤243	27/87 (31.0%)	1	0.2–1.1	0.041	8/87 (9.2%)	–	–	–
>243	2/18 (11.1%)	0.2			0/18 (0%)	–	–	–

Table 6. Adverse drug reactions during induction therapy and in the maintenance group

Adverse event	Induction instillations				Maintenance instillations				*P* value
	Frequency (%)	Grade (%)			Frequency (%)	Grade (%)			
		G 1	G 2	≥G 3		G 1	G 2	≥G 3	
Pain on urination	13/40 (48.1)	12	1	0	12/40 (30.0)	12	0	0	0.809
Urinary frequency	20/40 (50.0)	20	0	0	12/40 (30.0)	12	0	0	0.068
Gross hematuria	19/40 (47.5)	19	0	0	13/40 (32.5)	13	0	0	0.171
Difficulty with urination	17/40 (42.5)	17	0	0	15/40 (37.5)	15	0	0	0.765
All local events	23/40 (57.5)	22	1	0	27/40 (67.5)	27	0	0	0.657
High fever (>38°C or higher)	10/40 (25.0)	10	0	0	7/40 (17.5)	7	0	0	0.412
General malaise	0/40 (0.0)	0	0	0	2/40 (5.0)	2	0	0	0.152
Arythritis/arthralgia	0/40 (0.0)	0	0	0	1/40 (2.5)	1	0	0	0.314
Muscle pain	1/40 (2.5)	1	0	0	0/40 (0.0)	0	0	0	0.314
BCG sepsis	0	0	0	0	0	0	0	0	0
All systemic events	11/40 (27.5)	11	0	0	9/40 (22.5)	9	0	0	0.606

Many different maintenance schedules have been used. However, the optimal number, duration and total dosage of maintenance instillations remain empirical. Maintenance therapy consisting of a total of eight instillations every 3 months (13), a total of 12 monthly instillations (14), a total of 24 monthly instillations (15) and 6-week maintenance every 6 months for 2 years (16) were all reported to have no effect on prevention of recurrence. As a prospective study from SWOG (8) including 550 patients randomized to a weekly BCG regimen for 6 weeks, followed or not by three weekly instillations at the third and sixth months and every 6 months thereafter for up to 3 years, added support to the use of maintenance therapy and seems to be regarded as the standard maintenance instillation method, we followed this schedule in this study. To date, however, there is no universal consensus on the most effective instillation. From the results of univariate analysis in this study, the each respective institutions at 6, 12, 18 and 24 months as maintenance therapy are not significant prognostic factors for recurrence, individually. So, we examined the optimal total number and the total dosage of maintenance BCG instillations (Table 5). As a result, in terms of the total number of maintenance BCG instillations, compared with less than 4 times, maintenance BCG instillation of 4 times or more are significantly improve the tumor recurrence (HR 0.2, $P = 0.039$, Table 5). In terms of the total dosage of maintenance BCG instillations, compared with 243 mg or less, maintenance BCG instillation with a total dosage of >243 mg significantly improve the tumor recurrence (HR 0.2, $P = 0.041$, Table 5). No cases with maintenance BCG therapy of four times or more or with a total dosage of >243 mg as maintenance BCG therapy show progression (Table 5). We therefore concluded that maintenance BCG instillations for a total of four times or more or with a total dosage of >243 mg

after 6 months of induction therapy is necessary to obtain the optimal effect as maintenance therapy. In this study, we use the BCG Connaught strain with the standard dose of 81 mg. To paraphrase our results, therefore, when using the BCG Connaught strain, we should administered the standard dose of 81 mg four times or more as maintenance therapy. Needless to say, we need to be aware of the limitations of this study. After induction therapy, many patients want to delay or refuse the next instillation as maintenance therapy as much as possible. It is due in large part to a low frequency of maintenance instillation. Consequently, there is a possibility of insufficient evaluation as maintenance therapy. In their meta-analyses, Böhle et al. concluded that at least 1 year of maintenance BCG was required to show the superiority of BCG over mitomycin C in preventing recurrence or progression (3,17). Combined with our results it is necessary to examine in detail the total number, period and dose of instillations as maintenance therapy is required.

It is well-known that BCG intravesical instillation is often associated with severe ADRs. Several studies have investigated dose reduction of BCG, mainly for the purpose of decreasing the frequency and severity of these ADRs. Previously, several studies concluded that a one-third dose is as effective as a full dose but has less toxicity (18–20). In this study, there was no significant difference in the RFS between the full-dose instillation group and the dose-reduction group (data not shown). Accordingly, considering our results, when we reduce the of a single dose as maintenance instillation, we may expect the effect similar to standard dose by increasing the total number of doses.

Compliance with maintenance therapy is a serious issue (21). The completion rate of maintenance therapy in the SWOG trial was 16% (8), whereas another study (22) reported that only 1 of 111 patients completed seven cycles of maintenance therapy. Of the 65 patients on the maintenance therapy schedule of 6 weeks every 6 months for 2 years, 33.85% completed the planned 2-year treatment (16). In the present study 12 of the 40 patients (30.0%) completed 5 cycles of maintenance therapy. Despite a low rate of completion in the SWOG trial as well as in this study, BCG maintenance therapy significantly improved the rates recurrence and progression of high-risk NMIBC. That is to say, there may be many unnecessary instillations. Taken together including our results, it may be possible to considerably reduce the frequency of unnecessary BCG administration as maintenance therapy. Of course, since each patient has their own immune statuses, it may be difficult to establish the optimal instillation schedule for maintenance therapy. However, even a few local and systemic ADRs have a considerable influence upon a patient's quality of life. Our results show that it might be possible to decrease the number of maintenance instillations. To avoid unnecessary maintenance instillations and to reduce ADRs, establishment of an optimal maintenance schedule should be given high priority.

In conclusion, for prevention of recurrence and progression of NMIBC, maintenance BCG therapy was more effective than induction BCG therapy alone. Although limited in size and scope, the present retrospective study suggests both the usefulness of BCG maintenance therapy in patients with high-risk NMIBC and the probability that a schedule with fewer maintenance instillations would still be effective. The aim of future studies will be to continue exploring both the benefits and the optimal treatment schedule of maintenance BCG therapy in patients with high-risk NMIBC.

Conflict of interest statement

None declared.

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Testosterone augments polyphenol-induced DNA damage response in prostate cancer cell line, LNCaP

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Recently, we reported that combined ingestion of soy isoflavones and curcumin significantly decreased the serum level of prostate-specific antigen based on a randomized placebo-controlled double-blind clinical study. We investigated whether these polyphenols inhibited the proliferation of prostate cancer cells by activating a DNA damage response. The effects of isoflavones and curcumin on the expression and phosphorylation of ataxia-telangiectasia-mutated kinase (ATM), histone H2AX variant (H2AX) and checkpoint kinase2 (Chk2) were examined in LNCaP cells. The induction of apoptosis in LNCaP cells was evaluated by poly(ADP-ribose) polymerase (PARP) cleavage. Furthermore, the effects of a testosterone supplement on modulation of the DNA damage response were examined. Combined treatment of isoflavones and curcumin additively suppressed cellular proliferation and induced phosphorylation of ATM, histone H2AX, Chk2 and p53. Testosterone augmented the activation of the DNA damage response and PARP cleavage induced by curcumin. Our results indicate that activation of the DNA damage response by polyphenols might suppress the malignant transformation of prostate cancer. In addition, testosterone, when combined with curcumin, may have suppressive effects on the progression of prostate cancer. (*Cancer Sci* 2011; 102: 468–471)

While prostate cancer is the most common neoplasm in Caucasian men, the incidence in Asians has been relatively low. Observational studies have suggested that diet is one of the most important factors contributing to the lower observed incidence and mortality of prostate cancers in Asia.[1] Asian diets are rich in polyphenols, which have been suggested to prevent cancers.[2,3] Indeed, epidemiological studies have shown that soy intake is one of the major factors in the prevention of prostate cancer.[1] Polyphenols such as soy isoflavones and curcumin are common compounds in Asian diets, and both have anti-inflammatory and antioxidant properties. Previous studies show that curcumin and isoflavones induce apoptosis and cell cycle arrest in both androgen-dependent and androgen-independent prostate cancer cells.[4–6] Recently, we reported that a combined treatment of soy isoflavones and curcumin decreased serum levels of prostate-specific antigen (PSA) in subjects with a baseline PSA of more than 10 ng/mL in a randomized placebo-controlled clinical trial.[7] Furthermore, a combined treatment of soy isoflavones and curcumin inhibited the production of PSA and expression of the androgen receptor in cultured prostate cancer cells.[7]

The DNA damage response (DDR) emerges as an oncogene-inducible biological barrier against progression of cancer beyond its early stages. Recent evidence from cell culture and animal models and analyses of clinical specimens show that the early precursor lesions commonly express markers of an activated DDR.[8,9] These markers include phosphorylated kinases (ataxia-telangiectasia-mutated kinase [ATM] and checkpoint kinase2 [Chk2]), phosphorylated histone H2AX and phosphorylated p53. Such activation of the DDR network leads to senescence or death of oncogene-transformed cells that delays or prevents cancer progression. The DDR can also be induced by chemotherapeutic agents, UV and oxidative stress.[10] However, little is known about the effects of polyphenols, such as curcumin and isoflavones, on the induction of the DDR.

Androgens drive both the proliferation and differentiation of developing prostate epithelial cells. The maintenance of prostate epithelium requires continuous physiological levels of androgens, which inhibits apoptosis.[11,12] However, serum and tissue testosterone levels decrease as individuals age. Recently, several lines of evidence suggest that decreases in testosterone level accelerate the progression of prostate cancer. Indeed, low testosterone levels have been shown to be associated with an advanced tumor stage at presentation, positive surgical margins, high Gleason scores and worse overall survival.[13–17] Intraprostatic dihydrotestosterone (DHT) levels were significantly reduced in males with high-grade (Gleason scores 7–10) carcinomas compared with males with low grade carcinomas (Gleason scores ≤ 6).[18] These observations suggested that testosterone might suppress the malignant progression of prostate cancer. However, the mechanisms underlying the inhibition of malignant transformation in prostate cells by testosterone have not been fully elucidated.

The purpose of this study was to determine whether curcumin and isoflavone induced a DDR in prostate cancer cells as a potential mechanism of cancer prevention. Furthermore, we examined whether testosterone modulated the DDR in prostate cancer cells to elucidate the role of testosterone in cancer progression.

Materials and Methods

Cell culture and reagents. A human prostate cancer cell line, LNCaP, was obtained from the American Type Culture Collection (Rockville, MD, USA). The cells were routinely maintained in RPMI 1640 supplemented with 10% FCS, 100 units/mL penicillin and 100 μg/mL streptomycin. Cells were cultured at 37°C in a humidified incubator with 5% CO_2. Curcumin (Medi Herb Inc., Bangalore, India) was dissolved in ethanol at a concentration of 10 mM and stored at −20°C. Isoflavones (Nichimo Co., Ltd, Tokyo, Japan) were dissolved in DMSO at a concentration of 20 mg/mL and stored at −20°C in the dark. For the androgen stimulation analysis, LNCaP cells were cultured in a serum-free medium for 2 days prior to the addition of various concentrations of DHT (Wako, Tokyo, Japan) or synthetic androgen R1881 (New England Nuclear, Boston, MA, USA). To block androgen signaling, LNCaP cells were cultured with Flutamide (Sigma-Aldrich Co., St Louis, MO, USA).

Cell proliferation assay. LNCaP cells were seeded in poly-D-lysine-coated 96-well plates (Nalge Nunc, Rochester, NY, USA) at a density of 1×10^4 cells/well in 100 μL tissue culture

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medium. Cells were allowed to attach for 24 h and then treated with either increasing concentrations of curcumin or isoflavones. Cell proliferation was assessed using a colorimetric proliferation assay using tetrazolium3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H* tetrazolium (MTS) using the CellTiter 96 Aqueous One Solution Proliferation Assay System (Promega, Tokyo, Japan) according to the manufacturer's instructions. After 48 h, MTS was added to the culture wells and the mixture was incubated for 60 min at 37°C. The absorbance at 490 nm was measured on a microplate reader.

Immunoblotting. Subconfluent LNCaP cells were treated with curcumin, isoflavones or a combination of the two agents. Cells were washed twice with cold PBS and then lysed in radio immuno precipitation assay (RIPA) buffer on ice for 30 min. The cell lysate was centrifuged at 18500*g* for 30 min at 4°C and the supernatant was collected. Protein concentrations were measured using a BCA protein assay kit (Pierce Inc., Rockford, IL, USA) according to the manufacturer's instructions. Protein samples were separated by SDS-PAGE and transferred onto a PVDF membrane (Millipore Inc., Tokyo, Japan). Immunoblotting was performed using rabbit anti-androgen receptor antibody (1:2000; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), mouse anti-prostate-specific antigen antibody (1:1000; DakoCytomation, Kyoto, Japan), rabbit anti-phospho-ATM (Ser1981) antibody (1:2000; Rockland Immunochemicals Inc., Gilbertsville, PA, USA), rabbit anti-phospho-Chk2 (Thr68) antibody (1:1000; Cell Signaling Technology Inc., Danvers, MA, USA), rabbit anti-phospho-p53 (Ser15) antibody (1:1000; Cell Signaling Technology), mouse anti-p53 (DO7) antibody (1:1000; DakoCytomation), rabbit anti-phospho-H2AX (Ser139) antibody (1:1000 dilution; Upstate Inc., Temecula, CA, USA), rabbit anti-PARP antibody (1:1000 dilution; Cell Signal Technology) or mouse anti-human β-actin antibody (1:10 000 dilution; Sigma–Aldrich Co.) as an internal loading control. Immunoreactive proteins were visualized with ECL detection reagents (GE Healthcare Biosciences, Tokyo, Japan). The results of western blotting were quantified by densitometric analysis using a LAS 3000 Densitometer and Multi Gauge v3.1 (Fujifilm, Tokyo, Japan). The results were normalized to β-actin values.

Results

Combined inhibitory effects of isoflavones and curcumin on prostate cancer cell proliferation. We examined the effects of isoflavones and curcumin on the proliferation of LNCaP prostate cancer cells that express androgen receptors. Both isoflavones and curcumin inhibited the cell growth of LNCaP cells in a dose-dependent manner. Figure 1A shows that the combination of isoflavones and curcumin had a more potent inhibitory effect on cell proliferation than isoflavones alone. The cell viability in LNCaP cultures decreased by 42.5% when 20 μM curcumin and 20 μg/mL isoflavones were added to the culture medium. Figure 1B shows that 30 μM curcumin has an inhibitory effect on the cell growth of LNCaP in the presence of 100 nM DHT.

Activation of the DDR by isoflavones and curcumin. To determine the mechanism by which isoflavones and curcumin inhibit cell proliferation, we examined the phosphorylation of ATM, Chk2, histone H2AX and p53 by immunoblotting in cultures treated with polyphenols. The LNCaP cells were treated with 10 μg/mL isoflavones, 25 μM curcumin and a combination of these polyphenols for 48 h each. Phospho-specific antibodies that recognized each protein were used for this analysis. Isoflavones at a concentration of 10 μg/mL inhibited the expression of androgen receptors but did not induce phosphorylation of ATM, Chk2, histone H2AX or p53 in the LNCaP cells. Treatment with 25 μM of curcumin induced phosphorylation of these proteins. Moreover, a combination of the two compounds induced higher levels of ATM and Chk2 phosphorylation than curcumin only

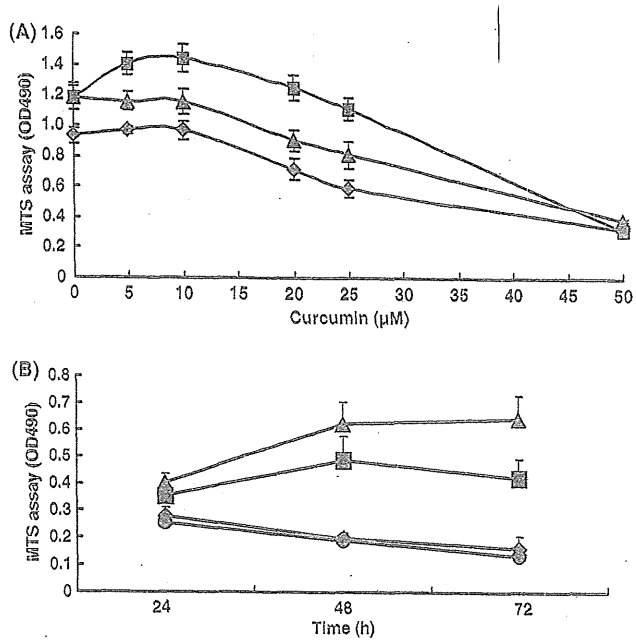

Fig. 1. (A) Effects of isoflavones and curcumin on the growth of LNCaP cells. Cells were treated with either increasing concentrations of curcumin and isoflavones. Varying concentrations of isoflavones, 0 μg/mL (■), 10 μg/mL (▲) and 20 μg/mL (◆), were added to the culture medium. After culturing for 2 days, cell viability was examined by MTS assay. Each point represents mean ± SD for six wells. (B) Curcumin has an inhibitory effect on cell viability of LNCaP even if the culture medium contained dihydrotestosterone (DHT). None (■), curcumin 30 μM (◆), DHT 100 nM (▲), curcumin 30 μM + DHT 100 nM (●).

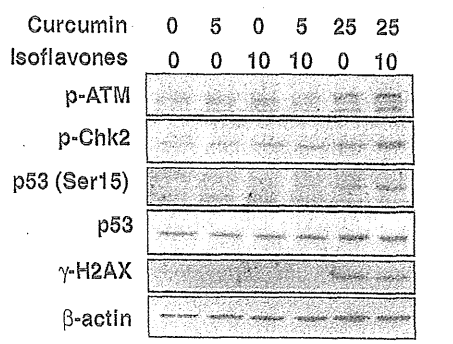

Fig. 2. Isoflavones and curcumin synergistically induce phosphorylation of ataxia-telangiectasia-mutated kinase (ATM), checkpoint kinase2 (Chk2), p53 (Ser15) and H2AX in the LNCaP cells. Protein expression was detected by immunoblotting after 48 h treatment. Immunoblots were probed for mouse anti-human β-actin antibody as an internal control.

(Fig. 2). Densitometric analysis showed that combined treatment of 10 μg/mL isoflavones and 25 μM curcumin induced the phosphrylation of ATM (fivefold), Chk2 (1.4-fold), p53 (63-fold) and histone H2AX (60-fold), respectively, when compared with the control.

Testosterone induced activation of the DDR in curcumin-treated cells. We showed that the proliferation effects of DHT were completely blocked by curcumin. Therefore, the effect of curcumin on the proliferation of LNCaP cells might be enhanced by testosterone through a DDR; this possibility was examined by western blotting analysis. For the androgen stimulation