Phase 2 Study of Adjuvant Intravesical Instillations of Apaziquone for High Risk Nonmuscle Invasive Bladder Cancer

K. Hendricksen,* E. B. Cornel, T. M. de Reijke, H. C. Arentsen, S. Chawla† and J. A. Witjes‡

From the Department of Urology, Radboud University Nijmegen Medical Centre, Nijmegen (KH, HCA, JAW), Department of Urology, Twente Hospital Group, Hengelo (EBC), and the Department of Urology, Academic Medical Centre, Amsterdam (TMdR), The Netherlands, and Spectrum Pharmaceuticals, Inc., Irvine, California (SC)

Purpose: We studied the safety and efficacy of multiple adjuvant apaziquone instillations in patients with high risk nonmuscle invasive bladder cancer.

Materials and Methods: Patients with high risk nonmuscle invasive urothelial carcinoma of the bladder underwent transurethral resection of all bladder tumor(s), and received 6 weekly adjuvant intravesical apaziquone instillations of 4 mg in 40 ml. Patients with carcinoma in situ received 3 further maintenance instillations at months 3, 6 and 12. Followup consisted of cystoscopy, urine cytology and observation of adverse events every 3 months for 18 months.

Results: A total of 53 patients were enrolled in the study. Although all patients were high risk according to the definitions used when the study was initiated, according to most recent guideline criteria, 80% and 20% of these patients would now be considered intermediate and high risk for recurrence, and 50% and 44% would be considered intermediate and high risk for progression, respectively. Intent to treat analysis of 49 patients with papillary tumors showed recurrent tumors in 34.7% and 44.9% at 12 and 18 months, respectively. One patient had progression to T2 or greater urothelial carcinoma after 9 months. There were 4 patients with carcinoma in situ who had complete responses at 3 months but discontinued treatment due to cystitis, recurrent papillary disease, urinary incontinence and dysuria. Most other side effects were mild (grade 1 to 2).

Conclusions: Adjuvant intravesical instillations of apaziquone are generally well tolerated. The recurrence rates of 34.7% after 12 months and 44.9% after 18 months in these patients can be considered encouraging, and warrant further study.

Key Words: apaziquone; urinary bladder neoplasms; drug therapy; administration, intravesical

Abbreviations and Acronyms

AE = adverse event

BCG = bacillus Calmette-Guérin

CIS = carcinoma in situ

MMC = mitomycin C

NMIBC = nonmuscle invasive bladder cancer

TURBT = transurethral resection of bladder tumor(s)

UC = urothelial carcinoma

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Study received ethical committee approval by local and central institutional board at each of the participating hospitals.

* Correspondence and requests for reprints: Radboud University Nijmegen Medical Centre, Department of Urology, intern mail 659, PO Box 9101, 6500 HB Nijmegen, The Netherlands (telephone: 0031 24 36 16712; FAX: 0031 24 35 41031; e-mail: f.witjes@uro.umcn.nl).

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‡ Financial interest and/or other relationship with Spectrum Pharmaceuticals, Sanofi Pasteur, Telormedix, Endo Pharmaceuticals and Medical Enterprises Ltd Amsterdam.

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On average 70% of patients with bladder tumors present with nonmuscle invasive bladder cancer, of which approximately 70% are Ta lesions, 20% T1 lesions and 10% CIS.[1] Standard treatment for NMIBC is transurethral resection of all bladder tumors followed by adjuvant intravesical instillations. The type and schedule of adjuvant treatment chosen depend on the risk of recurrence and progression of NMIBC.[2,3] The European Organisation for Research and Treatment of Cancer (EORTC)-Genito Urinary group has developed risk tables to calculate probabilities of disease recurrence that range from 15% to 61% at 1 year, to 31% to 78% at 5 years, and for progression from less than 1% to 17% at 1 year, to less than 1% to 45% at 5

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years.[4] These high percentages of recurrence and progression illustrate that current treatment regimens are suboptimal, and demonstrate a clear need for more effective treatment of NMIBC.

Apaziquone (EO9) is a novel fully synthetic indoloquinone based bioreductive alkylating agent that is converted to a cytotoxic species after enzymatic activation.[5] The enzyme deoxythymidine-diaphorase has a prominent role in the activation of apaziquone under aerobic conditions, but apaziquone is also cytotoxic under hypoxic conditions. In preclinical studies the concentration of apaziquone needed to achieve 50% cell death was 6 to 78 times lower than the concentration of MMC, depending on the UC cell line used.[6] In a marker lesion study by van der Heijden et al patients with Ta-T1 NMIBC were treated with apaziquone (4 mg/40 ml) instillations for 6 consecutive weeks.[7] A promising histological complete response was achieved in 30 of 45 (67%) patients and local side effects were comparable to those of other chemotherapeutic instillations. Of the complete responders 49.5% were recurrence-free at 24-month followup with a median response duration of 18 months.[8]

In the present study the primary objective was to examine the overall response and time to recurrence of papillary tumors treated with 6 weekly apaziquone instillations 2 to 4 weeks after TURBT in patients with high risk NMIBC. The secondary objective was to observe the duration of response and safety of apaziquone instillations.

MATERIALS AND METHODS

This multicenter prospective phase 2 trial was conducted at 3 Dutch hospitals. After ethical committee approval by the local and central institutional board at each of the participating hospitals, the study was conducted in accordance with the ethical standards set in the Declaration of Helsinki, amended version 1989.

Patient Selection

Following the initial diagnostic evaluation patients underwent complete TURBT of all visible bladder lesions and biopsies from suspicious areas, along with a bimanual palpation with the patient under anesthesia. The absence of upper urinary tract tumor was confirmed by radiological imaging within 6 months before treatment. Patients with high risk nonmuscle invasive UC of the bladder, defined as T1,[9] or grade 2b and grade 3,[10] or multiple and highly recurrent tumors (3 or more recurrences in 24 months) or CIS were eligible for the study.[11] Patients were fully informed of the investigational nature of the study and a signed written informed consent was obtained. Exclusion criteria were muscle invasive disease, prior intravesical treatment 3 months or less, apaziquone treatment less than 12 months, women who were pregnant or lactating, and expected poor compliance with the protocol.

Treatment

The first apaziquone instillation was given a mean ± SD of 21 ± 3 days after TURBT. Patients received 6 weekly intravesical instillations of 4 mg apaziquone in 40 ml, retained in the bladder for 1 hour. Patients with CIS received further maintenance therapy consisting of 3 weekly apaziquone instillations at 3, 6 and 12 months from the date of histological diagnosis.

Patient Evaluation and Followup

Followup consisted of toxicity evaluation, urine cytology, urinalysis and cystoscopy every 3 months for 18 months of followup. Adverse events were classified and graded according to the NCI-CTC (National Cancer Institute Common Toxicity Criteria) Version 3.0. Vital signs and Eastern Cooperative Oncology Group performance status were registered before each instillation. Serum chemistry and hematology were performed before the first and the fourth instillation. If study medication was withheld for 21 days or more due to intercurrent illness or AE, patients went off study. In case of suspicion of tumor recurrence, biopsies and/or TURBT were performed, and in case of recurrence, patients were discontinued from the study. Patients with CIS at study entry underwent urinary cytology and bladder biopsies at 3 months. If both were negative, patients continued with maintenance therapy, and followup during the remaining visits was by urine cytology and cystoscopy only. If the biopsy or cytology was positive, patients were discontinued from the study.

Pharmacokinetics

Pharmacokinetic investigations were performed in the first 10 patients treated in Nijmegen at apaziquone instillations 1, 4 and 6. This was done after obtaining an additional informed consent. Blood samples (3 ml) were drawn before the start of the apaziquone instillation (time zero), and 5, 10, 20, 40, 60 (the time of apaziquone drainage) and 75 minutes (15 minutes after voiding) after instillation. Measurements of apaziquone (EO9) and its metabolite EO5a were performed by a validated liquid chromatography/mass spectrometry method (Slotervaart Hospital, Amsterdam, The Netherlands).[12]

Statistical Considerations

We set a 50% desirable response rate at 18 months, ie a proportion of disease-free patients suggesting that the drug could be as active as the presently available drugs. With a power of 80%, α error of 5% and β error of 20%, and to reject with the same power an undesirable response rate of less than 30%, the minimum number of patients to recruit was 44. With an estimated dropout rate of 10% to 20% at 18 months, 53 patients were planned to be enrolled. Intent to treat analysis was performed on all patients receiving at least 1 apaziquone instillation.[13] The recurrence rate was defined as the percentage of patients with histologically proven recurrence at followup at a certain point. Disease progression was defined as the occurrence of UC stage T2 or greater.

To be able to compare our study results with the EORTC risk tables,[4] published after initiation of this study, we retrospectively studied the files for tumor size at study entry and previous intravesical therapies. With these additional

data we calculated the individual EORTC risk scores for recurrence and progression in our study cohort.

RESULTS

Patient and Tumor Characteristics

A total of 53 patients were enrolled in this study, including 40 males and 13 females, 51 white and 2 black. The age range was 37 to 85 years (mean 67.5). Table 1 includes the 6 prognostic factors and their accompanying weights according to EORTC risk tables[4] as well as prior adjuvant intravesical therapy. Tables 2 and 3 include the calculated recurrence and progression scores, respectively.

Safety and Tolerability

Of the 53 patients 51 received all 6 weekly instillations, 1 received 4 doses and discontinued due to pollakisuria, and 1 withdrew informed consent after 3 doses. All doses were 4 mg as planned, except for 1 patient who received 2 reduced doses of 2 mg due to urinary frequency. Eleven patients had a dose postponed, including 8 due to an AE, 1 due to patient wishes, 1 because catheterization was not possible and 1 due to abnormal urinalysis.

In total, 322 instillations were given and 49 patients (92%) experienced 194 AEs at a certain point. AEs reported by 10% or more of patients were dysuria (34%), hematuria (28%), urgency (23%), pollakisuria (21%), urinary tract infection (13%, not specified according to NCI-CTC), bacterial cystitis (11%) and fatigue (11%). Most AEs were grade 1 or 2. Allergic reactions were seen in 5 patients, including genital pruritus in 3 (grade 1), rash of the foreskin in 1 (grade 1) and erythema of the skin in 1 (grade 2). Grade 3 AEs were hematuria, urgency, pollakisuria, nocturia, urge incontinence, bacterial cystitis, pyrexia, lower abdominal pain, hypertension, decreased weight and atrial fibrillation, all reported once. Four patients (of whom 3 had CIS) discontinued the study due to AEs of pollakisuria, dysuria, chemical cystitis and urinary incontinence. Of these AEs only pollakisuria was considered treatment related. The only grade 4 AE was a myocardial infarction, which was considered unrelated. There were no notable changes in mean or median laboratory parameter values during the 4-week interval, and there were no notable changes in mean vital signs or performance status through week 6.

Pharmacokinetics

A total of 232 blood samples were collected (30 before and 202 after instillation). Apaziquone was detected (36.1 ng/ml) in only 1 sample at 5 minutes after the start of the instillation and EO5a was not detected in this sample. Neither apaziquone nor EO5a was detected in the remaining 201 plasma samples. The molar recovery percentage from the urine was high (mean 77%).

Table 1. *Six prognostic factors in accordance with EORTC risk tables and prior adjuvant intravesical therapy*

	Recurrence Score Weight	Progression Score Weight	No. Pts	Mean (range)
No. tumors:				
Single	0	0	19	
2–7	3	3	28	2.9 (1–10)*
8 or More	6	3	4	
Tumor size (cm):				
Less than 3	0	0	34	Not applicable*,†
3 or Greater	3	3	17	
Prior recurrence rate:				
Primary	0	0	20	
1 or Less/yr	2	2	9	4.1 (0–20)‡,§
Greater than 1/yr	4	2	23	
T category:				
Ta	0	0	41	Not applicable*
T1	1	4	10	
Grade:				
G1	0	0	4	
G2	1	0	32	Not applicable*
G3	2	5	15	
Concomitant CIS:				
No	0	0	49	Not applicable*,‖
Yes	1	6	2	
Prior adjuvant intravesical therapy:				
No therapy			23	0
Chemotherapy			9	1.3 (1–2)¶
BCG			3	1.7 (1–2)¶
BCG + chemotherapy			18	4.7 (2–10)¶

* Two patients had pure CIS only on biopsies.
† It was possible to estimate the maximum tumor size as less than 3 cm, or 3 cm or greater, but not to measure every single tumor.
‡ Number of previous occurrences.
§ Not known for 1 patient.
‖ Only 2 of the 4 patients with CIS had concomitant CIS.
¶ Number of courses of adjuvant intravesical therapy.

Table 2. *Calculated recurrence scores in accordance with EORTC risk tables*

Recurrence Score	Recurrence Risk Group	Probability of Recurrence at 1 Yr (%)	No. Pts	Mean Risk Score (range)
0	Low	15	0	6.7 (1–14)
1–4	Intermediate	24	14	
5–9	Intermediate	38	26	
10–17	High	61	10	

Table 3. *Calculated progression scores in accordance with EORTC risk tables*

Progression Score	Progression Risk Group	Probability of Progression at 1 Yr (%)	No. Pts	Mean Risk Score (range)
0	Low	0.2	3	6.5 (0–20)
2–6	Intermediate	1	25	
7–13	High	5	19	
14–23	High	17	3	

Efficacy

Intent to treat analysis was performed in 49 patients with papillary tumors. Recurrence rates at months 3, 6, 9, 12, 15 and 18 were 14.3%, 26.5%, 30.6%, 34.7%, 40.8% and 44.9%, respectively. In this group 1 patient had tumor progression from TaG2 to T2G3 or greater 9 months after TURBT. Three patients who were included in the study with a Ta tumor had a recurrence as T1. Two patients who were included with a T1 tumor had disease recur as Ta. All 4 patients with CIS had a complete response by histology at 3 months, but discontinued the study after 6, 6, 9 and 12 doses of apaziquone (3, 3, 5 and 12 months after study inclusion, respectively) due to chemical cystitis, recurrent papillary disease (T1G3), urinary incontinence and dysuria, respectively.

DISCUSSION

In this trial we studied the safety and efficacy of multiple adjuvant intravesical instillations of apaziquone in patients at high risk for recurrence and progression of NMIBC. At the start of the study in 2005 the definition used for high risk NMIBC differed from the current definition.[3,11] Since 2006 the EORTC risk tables have shown that the most important prognostic factors each contribute with a different weight to the risk of NMIBC recurrence and progression (table 1).[4] Therefore, the risk group classification was separated for recurrence and progression, and the guidelines for treatment of NMIBC were changed accordingly.[2,3] With the current definitions 80% of the patients included in this study were at intermediate risk for recurrence. The corresponding predicted probability of recurrence according to the EORTC risk tables at 1 year would have been 24% to 61% (table 2). In this respect the observed recurrence rate of 34.7% in this study is in the lower range. Similarly according to current definitions 50% of the patients in this study had an intermediate risk of progression, resulting in a predicted probability of progression of 1% to 5% at 1 year (table 3). Only 1 patient in our study had progression (1.9%). All 4 patients with CIS had a complete response at 3 months but the group was too small to draw conclusions.

The predicted risks of recurrence and progression in the EORTC risk tables have some limitations. Although the analyses were based on a large number of patients (2,596), 20% of patients initially received no treatment, less than 10% received an immediate post-TURBT instillation of chemotherapy, a second look TURBT was not performed in high risk patients and BCG was given without maintenance instillations. Therefore, the predicted recurrence and progression rates in these tables might be higher compared to treatment according to the recommendations of current clinical guidelines.[2,3] However, in our study a second TURBT was also not a standard procedure, nor did patients receive a single chemo-instillation immediately after TURBT. Recent literature suggests that 1 immediate instillation may only be useful in patients with a low risk tumor[14–16] and not in higher risk patients receiving additional intravesical instillations. However, this discussion is under debate.[17] In all, although this obviously is not a randomized controlled trial or comparative study, the observed efficacy data are in the better region of the anticipated range.

Cystitis and hematuria are the most common local side effects of intravesical therapy, but these usually resolve within 48 hours after instillation.[18] Chemical cystitis has been reported in as many as 56% of patients treated with doxorubicin, 41% of those treated with MMC[19] and approximately a third of subjects treated with epirubicin.[20] It is the most common side effect of BCG, occurring in approximately 80% of patients.[21] In this study the incidence of bacterial and chemical cystitis was in a similar range, between 11% and 34%. Hematuria occurred in 28% of patients in this study compared to up to 40% of those treated with intravesical chemotherapy,[19] in 32.6% of patients in the apaziquone marker lesion study[7] and in up to 90% of patients treated with BCG.[18] More severe but less common is the local adverse event of a contracted bladder. Four patients discontinued the study because of side effects, of which 1 (pollakisuria) was considered treatment related. However, in all of these patients complaints resolved after stopping therapy. Finally, allergic reactions have been reported in up to 14.8% of patients treated with MMC and 7.5% of patients treated with epirubicin.[22] Genital pruritus was observed in 6.5% of patients in the apaziquone marker lesion study.[7] In this study allergic reactions occurred in 5 patients (9.4%). Of these reactions 4 may have been due to local irritation (genital pruritus and rash of the foreskin). In all, the number and degree of side effects seem to be mild and comparable to other chemotherapeutic drugs used for intravesical instillation.

As previously discussed, theoretically systemic toxicity of apaziquone would be unlikely.[7] The systemic toxicity depends on penetration and absorption of the drug through the bladder wall into the bloodstream. Apaziquone's molecular weight of 288 limits penetration through the bladder wall. Furthermore, if penetration occurs, apaziquone is rapidly removed from the bloodstream because of a short half-life (1 to 19 minutes).[23,24] In addition, apaziquone can be potentiated under acidic extracellular pH (for example pH 6.0) conditions, but it may lose activity in the bloodstream due to an increase in extracellular pH to 7.35 to 7.45.[25] In this

study apaziquone and/or EO5a were not detected in 201 of 202 (99.5%) plasma samples. In 1 plasma sample 36.1 ng/ml apaziquone was detected, for which we do not have an explanation. Given the fact that it was detected only at 1 point (5 minutes after the start of instillation), we believe that it is a spurious observation. It is important to note that we have not observed any systemic toxicity.

CONCLUSIONS

The study results show that adjuvant intravesical instillations of apaziquone are in general well tolerated and side effects seem comparable to those of other chemotherapeutic drugs used for NMIBC. According to more recent guidelines, our study population was not purely high risk. Instead, it had an intermediate to high risk of recurrence, with corresponding predicted probabilities of recurrence of 24% to 61% at 1 year according to the EORTC risk tables. We observed recurrences in 34.7% of patients after 12 months and 44.9% after 18 months. The study population also had an intermediate to high risk of progression, with corresponding predicted probabilities of progression of 1% to 5% at 1 year. Only 1 of 53 patients (1.9%) had progression. The results of this phase 2 study can be considered promising and further study of adjuvant intravesical instillations of apaziquone is warranted.

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