Paclitaxel-Hyaluronic Acid for Intravesical Therapy of Bacillus Calmette-Guérin Refractory Carcinoma In Situ of the Bladder: Results of a Phase I Study

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Purpose: Carcinoma in situ represents high grade anaplasia of the bladder mucosa. Intravesical immunotherapy with bacillus Calmette-Guérin is the gold standard treatment for patients with carcinoma in situ. Patients with carcinoma in situ refractory to bacillus Calmette-Guérin are candidates for major surgery such as radical cystectomy. We identified the maximum tolerated dose and the recommended dose, and evaluated the safety profile of paclitaxel-hyaluronic acid bioconjugate given by intravesical instillation to patients with carcinoma in situ refractory to bacillus Calmette-Guérin.

Materials and Methods: A total of 16 patients with carcinoma in situ refractory to bacillus Calmette-Guérin were enrolled in a phase I, open label, single institution study. A minimum of 3 eligible patients were included per dose level. Paclitaxel-hyaluronic acid solution (ONCOFID-P-B™) was administered for 6 consecutive weeks. The primary objective was to identify the maximum tolerated dose and the recommended dose. As secondary objectives the safety profile of ONCOFID-P-B, the pharmacokinetic profile after each instillation and the tumor response were also evaluated.

Results: No dose limiting toxicity occurred at any drug level evaluated. The plasma levels of the study drug were always below the lower limit of quantification at all tested doses after each instillation. A total of 11 adverse events were reported by 7 patients and 9 (60%) showed complete treatment response.

Conclusions: Intravesical instillation of ONCOFID-P-B for carcinoma in situ refractory to bacillus Calmette-Guérin showed minimal toxicity and no systemic absorption in the first human intravesical clinical trial to our knowledge. Finally, satisfactory response rates were observed.

Key Words: paclitaxel; hyaluronic acid; administration, intravesical; carcinoma in situ; urinary bladder

Abbreviations and Acronyms

AEs = adverse events
BCG = bacillus Calmette-Guérin
CIS = carcinoma in situ
CR = complete response
DLT = dose limiting toxicity
HA = hyaluronic acid
NMIBC = nonmuscle invasive bladder cancer
NR = no response

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Study received approval from the Ethical Committee of the Catholic University and the Italian Superior Institute of Health.

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Bladder cancer is the 4th most common genitourinary cancer in men and the 7th in women with an incidence of more than 70,000 new cases in the United States in 2010.[1] NMIBC accounts for 70% of newly diagnosed bladder cancers.[2] The primary treatment for NMIBC is transurethral resection.[3] Intravesical instillation has been used to decrease recurrence and progression to muscle invasive bladder cancer since the 1970s.[4] CIS represents high grade anaplasia and is considered a precursor of muscle invasive bladder cancer.[5] BCG is the gold standard treatment for CIS. De-

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spite BCG treatment 42% to 83% of patients with CIS associated with papillary tumors and 20% to 34% with primary CIS experience progression to muscle invasive disease.[5] Radical cystectomy is the subsequent treatment and is considered a major surgical procedure with a 1% to 2.5% mortality rate in major series.[6] Thus, more active drugs are needed to treat NMIBC.

Paclitaxel is a chemotherapeutic agent with a wide spectrum of proven antitumor activity as well as activity against muscle invasive bladder cancer.[7,8] Theoretically these characteristics make paclitaxel an attractive candidate for intravesical therapy for NMIBC. Unfortunately because of its lipophilicity and the higher dose used in the intravesical therapy setting, paclitaxel is considered a poor candidate for intravesical therapy because it penetrates the urothelium, potentially causing systemic toxicity. A previous experimental study demonstrated that the conjugation between paclitaxel and HA reverses the lipophilic properties, and enhances antitumor in vitro activity against bladder cancer and bladder biocompatibility in vivo through acquired hydrophilic characteristics. HA is a linear polysaccharide formed by alternating D-glucuronic acid and N-acetyl-D-glucosamine units. Thus, the chemical conjugation between paclitaxel and HA makes the active ingredient water soluble and easier to handle in a therapeutic context.[9]

The chemical conjugation was obtained in several steps. Paclitaxel (1 gm) was dissolved in CH2Cl2, and 591 mg N-(3-dimethylaminopropyl)-N-ethylcarbodiimide hydrogen chloride and 796 mg 4-bromobutyric acid were then added to the solution. Subsequently the solution was partitioned in water. After eliminating the carbodiimide and bromide residues, the reaction solvent was dried with anhydrous sodium sulfate and eliminated with a rotary evaporator. The intermediate product (1.11 gm) was added to a solution of 4.38 gm HA-thiobarbituric acid dissolved in 220 ml anhydrous N-methyl-2-pyrrolidone. After a 7-day reaction at room temperature the solution was supplemented with 8 ml saturated NaCl solution. After 1 hour 750 ml ethanol were slowly added drop by drop. The resulting product was filtered, dissolved in water and dialyzed. When the conductivity of the dialyzed solution was less than 10 microseconds, it was frozen and subsequently freeze-dried. Paclitaxel loading was analyzed by high performance liquid chromatography analysis. The new conjugate had high solubility in a glucose aqueous solution of 14.6 mg HA-paclitaxel product obtained by esterification with a substitution of 16.3% wt/wt dissolved in 1 ml 5% glucose in water. The solution, with a paclitaxel concentration of 2.38 mg/ml, was filtered through a 0.20 μm filter. In addition, the maximum solubility of product in a 5% glucose aqueous solution was identified. At a concentration of 32.8 mg HA-paclitaxel, a viscous solution was obtained with an equivalent paclitaxel concentration of 5.35 mg/ml.

Thus, paclitaxel-HA bioconjugate (ONCOFID-P-B) could be considered a newer anticancer agent. As a primary objective in this phase I study we identified the maximum tolerated dose and the recommended dose, and evaluated the safety and the toxicity profile of intravesical ONCOFID-P-B (supplied by Fidia Farmaceutici S.p.A., Italy). Tumor response was a secondary objective.

PATIENTS AND METHODS

Eligibility Criteria

All patients enrolled in the study had a histologically and cytologically confirmed diagnosis of bladder CIS. Proven evidence of BCG refractory CIS was required. Other eligibility criteria included age between 18 and 80 years old, women with menopause and performance status 0 to 1 according to the Eastern Cooperative Oncology Group. Specific exclusion criteria were known hypersensitivity to paclitaxel or 1 of its constituents, concomitant papillary tumors (Ta–T1), previous systemic chemotherapy or radiotherapy, previous intravesical immunotherapy less than 3 months before study entry, renal and hepatic function values exceeding 2 times the upper normal value, significant cardiovascular diseases, and pregnant, lactating or any other malignancy within 5 years of study entry. The Ethical Committee of the Catholic University and the Italian Superior Institute of Health approved the study protocol and consent, and all patients provided informed consent before trial enrollment. The study was performed in accordance with the Declaration of Helsinki with the applicable regulatory requirements and Good Clinical Practice.

Intravesical Instillation

The product was supplied in 100 ml glass vials containing 50 ml sterile aqueous isotonic solution in which 750 mg paclitaxel-HA bioconjugate and 2.5 gm glucose were dissolved. The concentration resulted in 15 mg/ml in glucosate solution 5%, which represents the highest concentration achievable because of the viscosity of the drug.

Drug Administration and Dose Escalation

ONCOFID-P-B solution was administered weekly in a 6-week course. The final solution was infused by a 16Ch Foley catheter during a 5-minute infusion and the dwelling time was 2 hours. The phase I trial started with the first dose level of 150 mg, equivalent to 30 mg paclitaxel as a single intravesical instillation. The next dose levels were 300, 450, 600 and 750 mg. Dose levels were planned according to the Eisenhauer scheme. A minimum of 3 eligible patients were treated at each dose level. Before escalating to the next dose level all 3 patients of the evaluated level completed the 6 administrations and no intrapatient dose escalation was allowed. A minimum of 15 patients was required to complete the 5 planned levels. Three patients were treated at each dose level, and between the

entry of a patient and the next at a given dose level there was a minimum of 7 days. In absence of DLT a dose escalation to the next dose level was performed in 3 following patients. In cases of DLT at a given dose level the study protocol allowed the recruitment of 3 additional patients at the same dose level.

Toxicity Evaluation

At the screening visit several items were completed including a checklist of inclusion and exclusion criteria, informed consent, demographic and lifestyle data, medical history, medication history, physical examination, vital signs and electrocardiogram. Pretreatment laboratory assessments included a blood count, blood biochemistry study, standard urinalysis and urine culture. Chest x-ray and imaging (urography, bladder ultrasonography, computerized tomography or magnetic resonance imaging) were performed within 60 days before the screening visit.

The pharmacokinetic analysis was performed at all evaluated doses. To evaluate the systemic absorption of paclitaxel bioconjugate during and after intravesical instillation 9 blood samples of 5 ml each were collected in heparinized tubes according to the time intervals T0 (before instillation), and 5, 15, 30, 45, 60, 90, 120 and 150 minutes after instillation. The content of paclitaxel bioconjugate in plasma was evaluated with validated high performance liquid chromatography (according to the Bioanalytical Method Validation Guidance for Industry) in 2 steps. 1) The plasma samples were previously well separated from endogenous compounds present in the biological matrix and 2) finally analyzed in comparison with standard solution of paclitaxel. According to the quantification limit achieved from analysis validation, paclitaxel was detected at concentrations ranging from 303.6 to 2,259 ng/ml for plasma. The lowest limit of quantification values (303.6 ng/ml) below the lower limit of quantification were considered not detectable.

At the final visit 1 week after the last treatment, information was collected including vital signs, physical examination, electrocardiogram, concomitant medications, blood count, blood biochemistry, standard urinalysis, urine culture, and eventual toxicity and AEs. A followup visit was performed 4 weeks after the last treatment to identify late AEs and/or toxicities. AEs were evaluated according to National Cancer Institute-Common Toxicity Criteria v.3.0 criteria.

Tumor Response

Before and after treatment all patients were evaluated by imaging (urography, bladder ultrasonography, computerized tomography or magnetic resonance imaging), urethrocystoendoscopy with the patient under anesthesia with cold biopsies of bladder mucosa and prostatic urethra, and voided cytology. Complete response was defined as negative posttreatment cystoscopy including biopsy of the urothelium and negative cytology. Nonresponse was defined as the persistence of histologically or/and cytologically proven neoplasia. Tumor progression was defined as muscle invasive tumor.

RESULTS

Patient Characteristics

A total of 16 patients with BCG refractory CIS, otherwise candidates for radical cystectomy, entered the study (table 1). Mean ± SD age was 63.8 ± 11.8 years (range 29.1 to 76.4). All patients were white males. Performance status (Eastern Cooperative Oncology Group score) was 0 (asymptomatic) for all patients. Mean followup was 12.2 months.

Toxicity

Of the 16 patients 15 completed the 6 weekly instillations and were evaluable. In the first cohort 1 patient (dose level 150 mg) was excluded from study after 5 instillations because of cardiovascular disease not related to the study treatment. All 5 dose levels were completed (ONCOFID-P-B solution from 150 to 750 mg). No DLT occurred at any level of study drug during the escalating phase of the study. A total of 11 AEs were reported by 7 patients, including 8 that were not serious and 3 that were serious. All 8 nonserious AEs were mild (6) or moderate (2) in intensity and the patients recovered completely. Four AEs were judged remote (not related) and 4 not valuable/unlikely, including cystitis (2), overactive bladder (1), fever (1), hypotension (1), atrioventricular block (1), sinus bradycardia (1) and nonspecific anomaly of T wave (of electrocardiogram, 1). Overall 2 patients reported 3 severe AEs. In the first cohort 1 patient (dose level 150 mg) experienced worsening atrial fibrillation (preexisting condition) and cardiocirculatory failure. The case of heart failure recovered completely and atrial fibrillation stabilized. The patient was withdrawn from the study. The relationship with the study drug was not evaluable. A patient in the second cohort (300 mg) had late gross hematuria as a result of bladder biopsies. This AE occurred 40 days after the end of the 6th instillation and was considered not related to the study drug. The

Table 1. *Baseline patient characteristics*

Mean kg wt (SD)	81.13 (8.49)	
Mean cm ht (SD)	174.25 (6.97)	
No. smoking habit (%):		
Never	2	(12.5)
Current	6	(37.5)
Ex	8	(50.0)
No. alcohol (%):		
Never	1	(6.2)
Occasionally	13	(81.2)
Regularly	2	(12.5)
No. coffee or tea (%):		
Never	1	(6.2)
Occasionally	10	(62.5)
Regularly	5	(31.2)

event was mild in intensity, lasting 2 days, and the patient recovered completely. No clinically significant changes were observed in laboratory values, vital signs or physical examinations in all patients. Thus, the recommended dose of ONCOFID-P-B was fixed at 600 mg once a week for 6 consecutive weeks.

Pharmacokinetics

A total of 807 plasma samples from 16 patients were analyzed. In terms of the plasma levels the results were below the lower limit of quantification at all tested points of each instillation visit. In a few cases paclitaxel-HA was measured into the plasma but the level was always below the lowest limit of quantification and, therefore, the paclitaxel concentration had to be considered not detectable. All plasma samples were below the level of detection.

Response

At 1 week (±3 days) after the last instillation all patients underwent urethrocystoendoscopy under anesthesia, and cold biopsies of abnormal looking bladder mucosa and prostatic urethra as well as voided cytology were performed. Tumor response to ONCOFID-P-B was a secondary end point of the study and was evaluated at all tested levels of paclitaxel-HA solution during the escalating phase of the study (table 2). Concerning oncologic outcome at the final visit 6 patients (40%) had persistent CIS while 9 (60%) were disease-free. No patients experienced progression to muscle invasion bladder cancer.

DISCUSSION

Safety, tolerability and maximum tolerated dose were the primary objectives of the study. In this phase I study ONCOFID-P-B intravesical therapy for CIS refractory to BCG demonstrated a satisfactory safety profile and appealing response rates.[9]

Table 2. *Tumor response to paclitaxel-HA solution*

	No. (%)
All pts:	
CR	9 (60)
NR	6 (40)
150 mg:	
NR	3 (100)
300 mg:	
CR	3 (100)
450 mg:	
CR	3 (100)
600 mg:	
CR	2 (66)
NR	1 (33)
750 mg:	
CR	1 (33)
NR	2 (66)

Intravesical BCG is the standard treatment choice for CIS.[10,11] Unfortunately overall up to 50% of patients treated with intravesical therapy will experience recurrence or progression.[11] When currently available intravesical agents fail to control disease the option most likely to improve patient survival is radical cystectomy. Thus, more powerful intravesical agents are needed to treat NMIBC. ONCOFID-P-B showed antitumor activity and no systemic absorption as demonstrated by in vitro and in vivo experimental studies.

Rosato et al showed that the peripheral plasma concentration of paclitaxel-HA bioconjugate was low even 2 hours after instillation.[12–14] A subsequent study performed by filling the bladder with 1 ml of a solution of 5 mg/ml in paclitaxel of paclitaxel-HA (approximately 25 mg/ml) for 120 minutes showed only negligible concentrations of the drug (less than 1%).[14] This finding was confirmed using scintigraphy and no radionuclide activity was detected outside the bladder.[15] Tringali et al described the results obtained from in vitro rabbit whole bladder as a model to investigate the urothelial transport of ONCOFID-P-B after intravesical administration.[16] In these experiments the paclitaxel was almost completely recovered in the intravesical bath solution (more than 98%) while only 0.4% of administrated paclitaxel-HA bioconjugate was detected outside the bladder. A further study addressed the cytotoxic effects in human bladder transitional cell carcinoma. The study results on histoculture of surgical specimens from 16 patients showed that a 2-hour paclitaxel treatment was sufficient to produce apoptosis in 70% to 90% of human bladder tumors.[17] Thus, treatment with the bioconjugate caused inhibition of tumor cell proliferation and a greater apoptotic effect in the more rapidly proliferating tumors.[18] Rosato et al showed that treatment with paclitaxel-HA solution or HA induces equal over expression of CD44 receptor compared to baseline conditions, and that the extent of increase in fluorescence is the same as for the bioconjugate and HA according to exposure time.[19] Finally, the chemical conjugation between paclitaxel and HA makes the active ingredient water soluble and, therefore, easier to handle in the intravesical setting, which requires higher doses of the drug in close contact with the tumor and the absence of subsequent systemic absorption.

These data prompted us to evaluate the role of the bioconjugate in the bladder cancer clinical setting and in candidates for radical surgery due to nonresponse to standard intravesical treatment. In this study no systemic absorption was detected. No serious AEs were related to the administered drug. No clinically significant changes were observed in laboratory values, vital signs or physical examinations of all patients. As far as

the nonserious AEs were concerned only transient overactive bladder and cystitis were potentially related to treatment.[20] Although oncologic outcome was a secondary end point, we observed that 60% of patients showed a complete response. No patients experienced progression to muscle invasive bladder cancer. Activity based on response was demonstrated at any drug dose but the study design did not determine whether a dose response relationship would exist.

CONCLUSIONS

Intravesical instillation of paclitaxel-HA solution for NMIBC was well tolerated and there were no local or systemic AEs related to the drug. The preliminary oncologic results are satisfactory considering the patients were was otherwise candidates for major surgery such as radical cystectomy. These findings prompt further investigations in controlled clinical trials.

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