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Intravesical Gemcitabine in Superficial Bladder Cancer: a Phase II Safety, Efficacy and Pharmacokinetic Study

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Abstract. *Background: We investigated the safety, efficacy and pharmacokinetics of the intravesical administration of 2000 mg gemcitabine once a week in the four weeks before transurethral resection of superficial bladder cancer (TUR), and in the four successive weeks. Materials and Methods: Nine patients with superficial transitional cell bladder carcinoma were studied. Two thousand mg of gemcitabine dissolved in 50 ml of distilled water were administered intravesically. The dwell time was 60 min. The pharmacokinetics of gemcitabine and its metabolite, 2',2'-difluorodeoxyuridine (dFdU), were studied in plasma and urine before and after TUR. Cystoscopy was repeated 30 days after completion of the TUR treatment and subsequently at time intervals of one or two months. Results: No systemic toxicity was noted, and only three patients displayed modest signs of local toxicity. One patient had recurrence 1 month after TUR, three between 3 and 6 months, and another three after 8, 11 and 18 months, respectively; two were recurrence-free after 21 and 22 months, respectively. The peak plasma concentrations of gemcitabine never exceeded 1000 ng/ml before TUR and 350 ng/ml after TUR, and declined rapidly. The plasma levels of dFdU were higher than those of gemcitabine, increased until 60 min and then declined little. Between 52% and 100% of the gemcitabine dose was present in voided urine. Conclusion: Intravesical gemcitabine, at the dose of 2000 mg, is well tolerated, is associated with minimal systemic absorption and has a moderate efficacy in the treatment of superficial bladder cancer.*

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Bladder cancer is one of the most common malignancies; it accounts for 6% of all cancer cases in men and for 3% in women (1). More than 70% of bladder cancers are superficial at initial diagnosis (1) and transurethral resection of the tumor (TUR) is the initial treatment for such patients. Following TUR, a muscle-invasive disease that requires more aggressive therapy occurs in 20% to 30% of cases (2, 3).

Intravesical immunotherapy with *bacillus Calmette-Guerins* (BCG) is, at present, the most effective treatment for the therapy of superficial bladder cancer (4-6), but is associated with serious morbidity and even mortality (6-8).

Intravesical instillation of chemical agents, which assures direct contact with the tumor and, due to the minimal absorption, has the advantage of allowing the use of high doses with minimal systemic side-effects, has resulted in a significant reduction in the risk of recurrence. Intravesical chemotherapy with thiotepa, mitomycin C, doxorubicin and epirubicin has been shown to achieve complete response ranging from 34 to 53% (4, 9-11). Therefore, there is the need for more effective chemotherapeutic agents for the intravesical treatment of superficial bladder cancer.

Gemcitabine (2',2'-difluorodeoxycytidine) is a pyrimidine analogue that has been shown to produce good response rates when given systemically for the treatment of metastatic transitional cell carcinoma (12-14). A preclinical study in beagle dogs demonstrated that intravesical gemcitabine is well tolerated and has no direct bladder toxicity at doses of up to 350 mg (equivalent to the 1000 mg/m^2 human dose) administered on alternate days three times/week for four weeks (15). A phase I trial of eighteen patients with superficial bladder cancer refractory to intravesical BCG showed that gemcitabine given twice weekly in the bladder for six weeks, at doses ranging from 500 to 2000 mg, was associated with minimal bladder irritation and tolerable myelosuppression, and produced a complete or a mixed response in eleven patients (16). In a second phase I study, fifteen patients with recurrent superficial bladder carcinoma were treated once

Table I. *Patient characteristics.*

No of patient	Age and gender	Tumor stage	Local toxicity	Time interval without recurrence (months)
1	44 m	TaG1	absent	22
2	55 m	TaG1-G2	urinary frequency	6
3	63 m	TaG2	absent	5
4	73 m	TaG2	hematuria	21
5	71 m	TaG2	absent	18
6	72 m	TaG1	urinary frequency	8
7	60 m	TaG2-G3	absent	3
8	65 m	TaG1-cis	absent	1
9	72 f	TaG1-G2	absent	11

weekly for six consecutive weeks with intravesical doses of gemcitabine ranging from 500 to 2,000 mg.

Gemcitabine was detectable (≤1 μg/ml) only in the plasma of four patients receiving 2,000 mg in 50 ml. It was well tolerated, and nine of thirteen evaluable patients were recurrence-free at six weeks (17). Similar results were provided by a third phase I study in ten patients given six weekly intravesical instillations of 1,000, 1,500 or 2,000 mg of gemcitabine; it confirmed that the systemic absorption was very limited, the side-effects minimal and reversible, and the preliminary results satisfactory (18).

On the basis of the results provided by the three phase I trials, we conducted a phase II trial to evaluate the pharmacokinetics and the efficacy of 2,000 mg gemcitabine, administered intravesically once a week, in the four weeks before TUR and in the four weeks after TUR, in patients with superficial bladder carcinoma resistant to first-line intravesical immunotherapy or chemotherapy.

Materials and Methods

Patients. Patients eligible for this non-randomized, open-label, phase II study had histologically confirmed diagnosis of stages Ta-T1 or G1-G2 transitional bladder cell carcinoma, with persistent or recurrent disease despite prior intravesical therapy. Additional eligibility criteria included: age ≥18 and ≤75 years; a Karnofsky performance status ≥70%; adequate marrow function as defined by absolute neutrophil count ≥1,500 /μl, platelets ≥100,000 /μl; normal renal (serum creatinine ≤1.5 mg/dl) and hepatic function (serum total bilirubin ≤1.5 mg/dl, serum AST and ALT ≤two times the upper normal limit); negative pregnancy test for all women of childbearing potential; and informed consent. Patients with at least one of the following criteria were excluded from the study: presence of other neoplastic pathologies with the exception of basal cell carcinoma of the skin and carcinoma *in situ* of the uterine cervix; other severe pathologies influencing survival; higher sensitivity to the drug under test. The protocol of the study was approved by the Ethics Committee of the S. Martino Hospital, Genoa, Italy.

Protocol therapy. The protocol therapy consisted of the intravesical administration of 2,000 mg gemcitabine that was performed once a week for four consecutive weeks before the TUR of the tumor, and once a week for four consecutive weeks after TUR. Gemcitabine was reconstituted and diluted in 50 ml of distilled water and the pH was adjusted to 5.5 with sodium bicarbonate to avoid cystitis resulting from the low pH of the reconstituted gemcitabine. The bladder was completely emptied by straight catheterization before the instillation, and the patients was instructed to hold the drug for one hour before voiding.

Pharmacokinetic studies. Pharmacokinetic studies were performed in each patient at the fourth dose of gemcitabine before TUR and with the first dose after TUR. Blood samples were obtained before instillation of gemcitabine and 30, 60 (time of voiding) and 120 min after instillation. Each time, 5 ml of blood were drawn into heparinized tubes that had been preloaded with 0.05 ml of a 1 mg/ml solution of the cytidine deaminase inhibitor tetrahydrouridine. The blood samples were centrifuged for 10 min at 1,000 x g, and the resulting plasma was frozen and stored at –20˚C until analysis. Urine was collected before instillation of gemcitabine and on voiding. The volume of voided urine was measured and recorded , and an aliquot was frozen and stored at –20˚C until analysis.

The concentrations of gemcitabine and of its inactive metabolite, 2',2'-difluorodeoxyuridine (dFdU), were determined with a validated high performance liquid chromatography assay (19). The precision and accuracy of the method were determined by performing replicate analyses of pooled samples of drug-free human blank plasma spiked with six concentrations of gemcitabine and of its metabolite dFdU, selected to span the range of the standard curve (50-2,400 ng/ml) and to include the limit of quantification (50 ng/ml). The same procedure was performed with pooled samples of drug-free human blank urine spiked with concentrations of gemcitabine and dFdU ranging from 25 to 400 μg/ml.

Due to the rather high frequency of recurrences observed in the course of the study, we deemed it useful to quantify the uptake of gemcitabine in tumor cells. One g of a tumor resected from a patient not exposed to gemcitabine was incubated at 37˚C for 1 h in 5 ml of the same solution of gemcitabine (40 mg/ml) instilled in the bladder, and then divided into two fragments of the same weight. One of the two fragments was briefly washed with 3 ml of saline to remove the solution of gemcitabine. The second fragment was first washed for 2 min with 3 ml of distilled water to lyse erythrocytes which bind gemcitabine to a relevant extent, and subsequently washed 3 times for 2 min with 0.9% NaCl solution. The two fragments were homogenized with a potter in 1 ml of 0.9% NaCl solution and extracted as the plasma samples for HPLC analysis.

Results

Clinical and response characteristics. Between May 2002 and March 2003, nine patients, eight males and one female, 44 to 73 years of age (mean age 63.9±9.7 years), all with superficial transitional cell carcinoma of the bladder, were enrolled in the study (Table I). Intravesical administration of 2000 mg gemcitabine was performed once a week for 4 consecutive weeks, both before and after TUR. Patient no. 7 was not given the second course of gemcitabine.

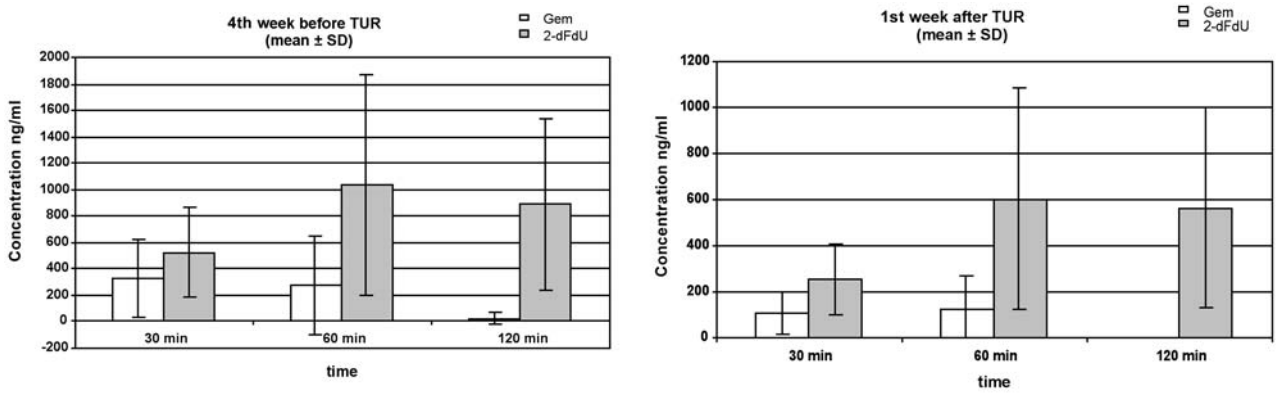

Figure 1. *Plasma concentrations (mean±SD) of gemcitabine* □ *and of 2-dFdU* ■ *at 30, 60 and 120 min after the fourth intravesical administration before TUR (9 patients) and the first administration after TUR (8 patients).*

Intravesical gemcitabine was well tolerated by all patients. Patient no. 2 suffered urinary frequency during the first course; patients no. 4 and 6 experienced hematuria and urinary frequency, respectively, during the second course.

Cystoscopy was performed in all patients at 30 days after completion of the intravesical therapy, and at time intervals of 1 or 2 months during the subsequent follow-up. No evidence of recurrence was noted in two patients, at 21 and 22 months respectively. One patient was noted to have recurrence 1 month after TUR, three patients between 3 and 6 months, and another three patients after 8, 11 and 18 months, respectively.

Pharmacokinetic studies. The average plasma concentrations of gemcitabine and of its inactive metabolite dFdU in blood samples obtained 30 min, 1 h and 2 h after the fourth intravesical administration before TUR and after the first administration after TUR are indicated in Figure 1. Gemcitabine was present in the plasma of eight patients, and under the limit of detection in the plasma of one patient. The peak concentrations displayed a high interindividual variability, but never exceeded 1,000 ng/ml before TUR and 350 ng/ml after TUR. The plasma gemcitabine concentrations declined rapidly, even during the hour of residing in the bladder and, with two exceptions, no gemcitabine was detectable 2 h after intravesical administration. The plasma concentration of dFdU increased progressively during the first 60 min after treatment and, in some patients, was even higher 60 min after voiding; as for gemcitabine, a high interpatient variability was observed at its peak concentrations.

The percentage of the administered dose of gemcitabine recovered in voided urine ranged from 52% to essentially 100%, with the lowest recoveries being present in patients subsequently shown to have substantial postvoid residual volumes.

The experiment performed to examine the uptake of gemcitabime by tumor cells revealed that, in the fragment of tumor incubated for 60 min at 37˚C with the same solution of gemcitabine used for the intravesical administration (40 mg/ml), and subsequently washed once with distilled water to lyse erythrocytes and then three times with saline, the amount of gemcitabine was 0.39 ng/mg protein. This finding suggests that the uptake of gemcitabine by tumor cells was rather low.

Discussion

The results of this study raise a number of questions and, at the same time, provide suggestions relevant to the clinical application of intravesically administered gemcitabine. First of all, they indicate that the dose of 2,000 mg was associated with little absorption of gemcitabine from the bladder into the systemic circulation, and that its plasma concentrations, which displayed a marked interindividual variability, were transient. In fact, the plasma concentrations of gemcitabine had often already decreased during the time that gemcitabine was residing in the bladder, being in some patients higher after 30 min than after 60 min of its intravesical administration, and were drastically reduced or under the limit of detection 1 h after voiding. On the other hand, in the majority of patients the plasma concentrations of the inactive metabolite dFdU were higher than the plasma concentrations of gemcitabine 30 min after its intravesical administration, displayed a marked increase after 60 min, and were still higher than at 30 min one hour after voiding. Taking into account that theoretically the absorption of gemcitabine from the bladder should have taken place at a constant rate during the 60 min of residing in the bladder, and that the half-life of gemcitabine ranges from 40 to 90 min (20), the observed pharmacokinetic behaviour is difficult to explain. A possible hypothesis is that gemcitabine

absorption from the bladder occurs *via* an active process, and that saturation of membrane transporters may occur (21).

The high interpatient variability observed in the plasma concentrations of gemcitabine was found to be independent of both the volume and pH of the urine present in the bladder at voiding; it was rather greater before than after TUR, and this may be ascribed to the different size of the neoplastic lesions. After TUR, the absorption of gemcitabine was even lower and displayed a smaller interpatient variability. The lower absorption may be the consequence of the resection of the tumor and of the healing of the surgical wound in the bladder mucosa; this hypothesis is supported by the even lower absorption that occurred in patients who were given the first dose of gemcitabine after a time interval from TUR greater than 8 days.

It is important to underline that our findings about the absorption of gemcitabine from the bladder are in substantial agreement with those Laufer *et al.* (17) and of Witjes *et al.* (18), who did not formulate any satisfactory hypotheses about its unpredictable pharmacokinetic behaviour.

Concerning the efficacy of the regimen of gemcitabine administration utilized in this study with the aim of preventing recurrences, the cystoscopies performed during the period of follow-up revealed that one patient had recurrence one month after TUR and six patients between 3 and 18 months, whereas no evidence of recurrence was noted in two patients after 21 and 22 months, respectively.

Taking into account that the results of this study indicate that, with the present regimen, the intravesical administration of 2000 mg gemcitabine never resulted in systemic toxicity and produced signs of local grade 1 toxicity in only three patients, it seems reasonable to examine whether a more frequent administration of gemcitabine, *e.g.* alternate days, and/or a higher dosage, may assure a lower incidence of recurrences.

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