Pharmacokinetics and Toxicity of an Early Single Intravesical Instillation of Gemcitabine after Endoscopic Resection of Superficial Bladder Cancer

FABIO CAMPODONICO1, FRANCESCA MATTIOLI2, VALERIA MANFREDI2, GIACOMO CAPPONI1, PAOLO PASQUINI1, ANTONIETTA MARTELLI2 and MASSIMO MAFFEZZINI1

1Division of Urology, E.O. Ospedali Galliera, Genoa;
2Division of Clinical Pharmacology and Toxicology, University of Genoa, Italy

Abstract. Background: The tolerability and plasma absorption of gemcitabine administered at 40 mg/ml after small and extensive endoscopic transurethral resection of bladder tumors (TURB) were evaluated. Patients and Methods: Nine patients with a history of recurrent superficial bladder cancer were eligible for a single immediate, post TURB, intravesical instillation of gemcitabine. The endoscopic resection was small in 5 patients and extensive in 4. The drug was administered at 40 mg/ml concentration (2000 mg in 50 ml saline) and held in the bladder for 1 hour. Plasma concentrations of gemcitabine and its metabolite (2',2'-difluorodeoxyuridine) were determined with a validated HPLC assay. The blood count and chemistry were performed one day and one week postoperatively. Results: Toxicity was comparable for patients who underwent small or large TURB. The most significant side-effects were grade 2 vomiting and a transient grade 2 leukopenia after small and large TURB respectively. Mean maximum gemcitabine concentrations were 1.47 ìg/ml in small TURB and 2.8 ìg/ml in large TURB. The highest peak concentration of 4.26 ìg/ml was found after extended bladder resection. Conclusion: A single, immediate postoperative, intravesical instillation of gemcitabine at high concentration is feasible with acceptable toxicity, and it may be considered as an option taking into account patient performance status, tumor characteristics and TURB extension.

The natural history of superficial bladder cancer shows a high rate of recurrence, around 80%, and a lower rate of stage-grade progression (1). The dilemma linked to the high recurrence is not only related to the potential polychronotopic development of tumors beyond the urothelium. Soloway and Masters first demonstrated that tumor cells preferentially implant (from four to five fold) on a cauterized urothelial surface and that instillation of thiotepa or mitomycin significantly reduced the incidence of implantation (2). In order to be effective, the intravesical instillation should be started within one hour of tumor resection, since it dramatically decreases the incidence of bladder and urethral implantation (3). These results suggest that cell seeding is a significant factor which increases the recurrence rate after transurethral resection of bladder tumors (TURB) and that intravesical chemotherapy initiated immediately after TURB may reduce cell implantation. Other studies have focused on optimizing the intravesical administration of chemotherapy after surgery. The variable drug sensitivity of bladder tumors is partly due to their intrinsic malignancy (stage and grade) and partly to inadequate drug delivery (4, 5). In an in vitro system with a human bladder cell line, the drug concentration and period of exposure showed an exponential relationship with clonogenic cell kill (6). Recently, in a randomized study, Au et al. optimized the intravesical chemotherapy by increasing dosage and concentration with an improved 5-year recurrence-free survival when compared to conventional treatment (7). Thus, an evident therapeutic benefit can be obtained by using the highest concentration achievable for as long as the patient can retain the drug in the bladder. The EAU Working Party on non-muscle invasive bladder cancer recommended an immediate single postoperative instillation with a chemotherapeutic drug as a standard treatment for low and intermediate risk tumors (8). Of the new drugs which demonstrated activity against superficial bladder cancer, gemcitabine has been demonstrated to be one of the most promising in phase II trials (9-11). Moreover, since gemcitabine displayed a rapid and consistent in vitro cytotoxic activity, it has been proposed as a potentially useful agent to prevent tumor cell reimplantation immediately after TURB (12).

The purpose of our study was to investigate the tolerability and plasma absorption of gemcitabine administered at
40 mg/ml (saturation dose) after small and extensive endoscopic resection of bladder tumors.

Patients and Methods

Eligibility criteria. Patients with a history of recurrent superficial bladder cancer were eligible for a single immediate, post TURB, intravesical instillation of gemcitabine. Additional criteria included informed consent, WHO performance status 0-2 and adequate bone marrow, hepatic and renal function. Patients were required to have no urethral stricture or bladder diverticula and should not have previously undergone prostate/urethral surgery or abdomen radiotherapy, which may change the structure of the bladder mucosa. Prior intravesical drug treatments were allowed, except for BCG in the last 6 months in order to avoid any concomitant cystitis.

Nine patients were divided into two groups depending on the volume of the tumor. Five patients had a limited disease resectable in a few loop passages not exceeding 6 resections (minimal TURB), and four patients had a greater tumor volume (large TURB). Any strong suspicion of or clear bladder perforation during TURB deemed patients unsuitable for drug instillation. The protocol was approved by the local Ethics Committee.

Chemotherapeutic treatment. Gemcitabine was prepared in solution as suggested by previous phase II studies (13). Two thousand mg of drug were diluted in 50 ml normal saline (0.9%) to achieve the saturation dose of 40 mg/ml. At the end of TURB, the bladder wall was meticulously examined paying particular attention to haemostasis and bladder perforation. Thus, the drug was instilled into the empty bladder and the catheter was closed for one hour.

All patients were operated on with epidural anesthesia and received restricted hydration with intravenous saline during the procedure.

Pharmacokinetic analysis. Blood samples were obtained before instillation of gemcitabine, and at 15, 30, 60 (time of voiding), 90 and 120 min after instillation. Each time, 4 ml of blood were drawn into heparinized tubes that had been preloaded with 0.04 ml of a 10 mg/ml solution of the cytidine deaminase inhibitor tetrahydrouridine (THU). Blood samples were centrifuged for 10 min at 1,000 xg, and the resulting plasma was frozen and stored at –20°C until analysis.

Concentrations of gemcitabine and its metabolite 2', 2'-difluoro(deoxy)uridine (dFdU) were determined with a validated high performance liquid chromatography assay (14). The chromatography system consisted of a 325 pump system, a 535 UV detector and signal integration software KromaSystem 2000 (BIO-TEK Instruments S.r.l., Milano, Italy). A 5 µm, 250x4 mm I.D. Adsorbosphere NH2 column (Alltech Italia S.r.l., Milano, Italy) with a 5 µm, 10x4.6 mm I.D. Adsorbosphere NH2 guard column were used to analyse samples. Fresh human plasma samples were obtained from healthy volunteers for standard samples. The precision and accuracy of the method were determined by performing replicate analyses of pooled samples of drug-free human blank plasma spiked with 2-deoxycytidine as internal standard, at concentration of 8 µg/ml, and six concentrations of gemcitabine and of its metabolite dFdU selected to span the range of the standard curve (0.05-2.4 µg/ml) and to include the limit of quantification (LOQ 0.05 µg/ml).

Two replicates of each plasma concentration level (0.05, 0.1, 1.0, 2.0 µg/ml), analyzed on three different days, were subjected to within and between run analysis. Samples with concentrations higher than the upper limit of the calibration were re-analyzed by dilution of the sample. The precision (RSD of replicate analysis) was calculated using the ANOVA test; the accuracy of the method was calculated by the formula: BIAS = (mean - nominal concentration)/(nominal concentration x100) (15).

The pharmacokinetic parameters calculated were: maximum plasma concentration (C max), area under the curve of plasma concentrations versus time extrapolated to infinity (AUC∞), elimination rate constant (K el) and half-life (t 1/2). C max was determined by visual inspection of each of the plasma concentration-time plots. AUC∞ was calculated on log-transformed values with the use of the trapezoidal rule and extrapolated to infinity by dividing the last measurable plasma concentration value by K el. K el was estimated from the points of the terminal phase of the plasma concentration curve by log-linear regression.

Toxicity evaluation. Toxicity was assessed following the Common Toxicity Criteria v 3.0 (16). Local side-effects, even when masked by peripheric anesthesia, were checked and recorded if found. The systemic toxicity was evaluated by clinical examination and after discharge by interview. The blood count and chemistry were performed pre-operatively, then one day and one week postoperatively.

Results

Patients were recruited between October 2005 and May 2006. All patients completed the procedure successfully. No significant hematuria was observed at reconnection of the catheter, 1 h after postoperative instillation of gemcitabine. The urinary volume collected at 1 h, ranged from 70 to 300 ml (mean 181 ml with SD 86 ml). The mean urinary pH of urine recovered at 1 h after intravesical instillation was 3.8 (ranging from 3 to 4, SD 0.4). Patient characteristics are listed in Table I.

Pharmacokinetic studies. The calibration curves of peak areas versus concentrations of gemcitabine and dFdU were linear giving a correlation coefficient r² of 0.999. The results as far as precision and accuracy are concerned, derived from the measured concentrations of the validation samples, were acceptable according to Washington criteria (17).
Plasma concentrations of gemcitabine and of its inactive metabolite dFdU in blood samples obtained 15, 30, 60, 90 and 120 min after intravesical administration are indicated in Table II. Pharmacokinetic parameters of gemcitabine are shown in Table III.

Gemcitabine was present in the plasma of all patients and below the detection limit in the plasma of three patients (patient 1, 4, 7) at 120 min and of one patient (patient 4) also at 90 min after intravesical instillation. Peak concentrations displayed a high interindividual variability but never exceeded 4.26 µg/ml. The highest gemcitabine concentrations were found in patients 8 (4.26 µg/ml = 14.21 µM) and 6 (3.33 µg/ml = 11.11 µM) who underwent large TURB, and in patient 4 (2.59 µg/ml = 8.64 µM). Mean maximum gemcitabine concentrations were 1.47 µg/ml in small TURB and 2.8 µg/ml in large TURB.

Plasma gemcitabine concentrations declined rapidly, even while being instilled in the bladder, as revealed by the average plasma concentrations (Figure 1). The plasma concentration of 2dFdU increased progressively during the first 60 min after intravesical instillation, and in all patients was still increasing 60 min after voiding. The mean levels of plasma 2dFdU in large and small TURB were statistically different (p<0.05) at 60, 90, 120 minutes, using a two-tailed independent sample t-test. As for gemcitabine, a high interpatient variability was observed in the peak concentration of the metabolite. In patients 4, 6 and 8, the highest levels of dFdU were found thus confirming the elevated absorption of gemcitabine. The percentage of the administered dose of gemcitabine recovered in voided urine ranged from 16% to 99%.

Systemic and local toxicity. In the group which underwent small TURB, the most significant side-effect was grade 2 vomiting in one patient which occurred one day after treatment. Other symptoms were vomiting and grade 1 dysuria in two patients. In the large TURB group, a grade 2 leukopenia in one patient and mild fatigue in another were observed. The leukopenia was probably transient since it had recovered at the following blood test one day later. Transaminases were normal in all blood tests. No patient experienced local side-effects such as bladder pain or burning during the interval of bladder retention or afterwards, but urinary symptoms appeared after the catheter was removed one/two days later. Therefore, as far as immediate local symptoms are concerned, there was no difference for patients operated on with general or with epidural anesthesia. All side-effects are reported in Table IV.

None of the nine patients had any recurrences at the 3-month cystoscopy check.

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SD = standard deviation; SE = standard error.

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Table II. Plasma concentration of gemcitabine and 2dFdU at 15, 30, 60, 90 and 120 minutes after intravesical administration.

Table III. Pharmacokinetic parameters of gemcitabine after intravesical instillation of 40 mg/ml.
Discussion

The study was conducted with the aim to investigate the administration of gemcitabine at high concentration (i.e. 2000 mg diluted in 50 ml normal saline) in bladders which had just undergone tumor resection. This dose, at a concentration of 40 mg/ml, is that recommended in previous phase I-II studies. These studies however, which demonstrated safety and substantial activity against superficial bladder cancer, were performed on intact bladders. Palou et al. (18) first investigated the use of gemcitabine as an early single instillation after TUR of superficial bladder cancer. The tolerability and pharmacokinetic analysis were assessed in 10 patients receiving gemcitabine at concentrations of 15 and 20 mg/ml. Blood samples were collected at several time intervals for 4 h after instillation. Plasma gemcitabine was low in all patients; the highest drug concentrations (6.1 and 4.5 µg/ml) were found in two patients in whom bladder perforation was strongly suspected. The maximum concentration of plasmatic gemcitabine occurred after a one hour retention time in 7 out of 10 patients. Similarly, the concentration of dFdU reached a long plateau after a steady increase. Even excluding the two patients with bladder perforation, who had an obvious high drug absorption, the differences between the other patients were still remarkable (up to 10-fold). Thus, the interpatient variability did not explain the relationship between the dose administered and plasma concentration. However, in this trial no patient experienced toxicity above grade 1, and the authors demonstrated the safety of a single post-TURB intravesical instillation of gemcitabine at low concentration.

In our study, two group of patients received an intravesical high concentration of gemcitabine but the resected surface of the bladder was different. The two highest plasma peaks of gemcitabine were found in patient 8 and 6 who underwent large TURB. By comparison, if we consider as topline the average peak concentration of 10 µg/ml achieved after intravenous administration (19), the mean peak of 2.04 µg/ml after intravesical instillation rated 20%, and the two peaks of patient 8 and 6 (4.26 and 3.33 µg/ml) rated 43% and 33%, respectively. Respectively, these two patients experienced a transient grade 2 leukopenia and a grade 1 fatigue which do not represent side-effects which would limit clinical application.

The kinetic curve of plasma gemcitabine was substantially different to that reported by Palou et al. (18). The highest mean plasma peak of all patients was immediately achieved at the first blood test after 15 min. Among patients who underwent large TURB, 3 patients out of 4 achieved the maximal plasma drug concentration at 15 min, while in the small TURB group only 1 patient out of 5 had a rapid plasma increase of gemcitabine. The slope of concentrations after maximum peak showed a steadily decreasing curve rather than a plateau. Similarly, the rise of plasma dFdU was in direct relation to the gemcitabine concentration, as expected when pharmacokinetic measures are consistent. In the study by Palou et al. (18), the slow rise and delayed concentration peak was due to the lower drug concentration at baseline, and should reflect a constant absorption throughout the intravesical retention time interval. Conversely, at 40 mg/ml gemcitabine concentration, despite an early plasma peak, the succeeding drug concentrations were somewhat lower. The most intuitive explanation may be the dilution of intravesical gemcitabine, because an initial small volume (50 ml) is rapidly diluted by urine production. The pharmacokinetic evaluation performed by other authors on intact bladder by using 40 mg/ml of gemcitabine showed an immediate plasma peak followed by a constant decrease in drug concentrations.
concentration, which is comparable to the trend of our results (20-22). Thus, it seems that the initial intravesical concentration may be the most relevant factor affecting the kinetics and absorption of gemcitabine rather than the extent of the damaged bladder surface after TURB.

Conclusion

In patients with superficial bladder cancer, an immediate intravesical instillation of gemcitabine at high concentration is feasible with acceptable toxicity. Although the activity of 40 mg/ml gemcitabine has only been investigated in trials on intact bladders, the urologist may consider, as an option, perioperative intravesical instillation at low or high drug concentration, taking into account all clinical aspects, such as patient age, performance status, tumor characteristics and TURB extent.

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References

16 http://ctepl.cancer.gov