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Pharmacokinetics study to optimize the intravesical administration of gemcitabine

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Running head: OPTIMIZATION OF INTRAVESICAL GEMCITABINE ADMINISTRATION

Abbreviations: TUR, transurethral resection; HPLC/HRMSn, high-pressure liquid chromatography/high-resolution mass spectrometry; dFdC, gemcitabine (2',2'-difluorodeoxycytidine); dFdU, 2',2'-difluorodeoxyuridine;

Abstract

Introduction To assess in a phase II pharmacokinetic study whether different pH levels, dilution volumes and exposure times affect intracellular bioavailability and systemic absorption of gemcitabine.

Subjects and Methods Six arms of three patients each with a non-muscle-invasive bladder cancer (NMIBC) were planned to receive six combinations of two different dilution volumes (50 mL vs 100 mL), two pH levels (2.5–3.5 vs 5.5) and two exposure times (1 h vs 2 h) of the study drug. Blood samples were taken before, during and 1 h after drug instillation. Cold biopsy specimens from the exophytic tumor, its base of implant and a macroscopically healthy mucosa were taken during transurethral resection. High-pressure liquid chromatography (HPLC)/high-resolution mass spectrometry (HRMSn) analysis of plasma and tissue samples was used to determine concentrations of gemcitabine (dFdC) and its inactive metabolite (dFdU).

Results The arm at pH 5.5 in 50 mL was withdrawn as 2000 mg dFdC are insoluble in these conditions. The different instillation conditions resulted in negligible plasma dFdC concentrations but significant differences in intracellular content and metabolism of dFdC. The lowest intratissue concentration of dFdC was detected in a 50 mL solution at a pH of 2.5–3.5 kept in the bladder for 1 h (standard arm). A pH 5.5 solution in 100 mL with a 2-h exposure favored the maximal intratumoral dFdC absorption which was 90 times higher than that recorded in the standard arm.

Conclusions The most commonly reported administration scheme of gemcitabine produced the lowest tissue bioavailability of dFdC. Other combinations of pH, dilution volume and duration of instillation proved more advantageous and merit testing in clinical trials.

Introduction

The search for intravesical agents with less toxicity and a better efficacy profile has prompted studies of novel chemotherapeutic options with effective antitumor activity and pharmacokinetic profiles suitable for regional therapy. Gemcitabine (2',2'-difluorodeoxycytidine, dFdC), a deoxycytidine analogue with a broad spectrum of antitumor activity, is an ideal candidate for this purpose. Gemcitabine is transported into the cell, phosphorylated and incorporated into DNA and RNA, causing cell growth inhibition and mediating apoptosis. The drug is then deactivated by deamination into 2',2'-difluorodeoxyuridine (dFdU) and transported out of the cell [1,2]. Its molecular mass of 299.7, which is lower than that of other commonly-used intravesical drugs, may enable the drug to penetrate the bladder mucosa; at the same time it could be high enough to prevent significant systemic absorption in an intact bladder. Phase I pharmacokinetic studies have shown
that systemic absorption is minimal when up to 40 mg/mL drug concentration is given intravesically [3–6], immediately after transurethral resection (TUR) [7]. Phase II marker lesion studies have shown interesting ablative response rates ranging from 22% to 67% [8,9], which are comparable to other commonly-used intravesical drugs [10], and a promising safety profile [3,4]. Reviewing the published phase I and II studies, wide discrepancies emerge in the instillation volume (100 mL [3,7,11] vs 50 mL [4–6,8,12–14]), the pH of the solution (a buffered solution at pH 5.5 [3,11], in contrast with the unbuffered one at pH 2.5–3.5 which is usually employed) and the instillation time (1 h [3,5–7,11,12–14] vs 2 h [4,6,8]). Most of these choices are not supported by a specified scientific rationale.

The present clinical pharmacokinetic study was designed to assess the intratissue penetration and systemic absorption of gemcitabine and its metabolites in different administration conditions to verify whether any of the above-mentioned parameters affect the systemic and tissue absorption of the drug. Such a study may also provide indirect information about how to optimally combine gemcitabine therapeutic efficacy with safety. In addition, the study may provide an experimental model that can be applied to traditional chemotherapeutic agents (Mitomycin C and epirubicine), the administration conditions of which are still mostly based on empirical grounds.

**Material and Methods**

The study was approved as a single centre, open label, non-randomized phase II trial by the Ethical Committee. The primary objective was to assess whether different conditions of dilution volume, pH of the solution and exposure time for the standard 2000 mg gemcitabine dose could affect its systemic absorption and the intratissue concentration of dFdC and its metabolites. Patient inclusion criteria were a cystoscopically proven primary or recurrent, solitary or multifocal non-muscle-invasive bladder cancer (NMIBC), an age ≥18, ECOG (Eastern Cooperative Oncology Group) performance status ≤2, adequate bone marrow function, renal function (serum creatinine less than two times the upper limit of normal), hepatic function (bilirubin, aspartate transaminase and alanine aminotransferase less than two times the upper limit of normal). Six combinations (arms) of the drug (2000 mg) differing in the dilution volumes (50 or 100 mL), pH of the solution (2.5–3.5 obtained by dissolving the drug in unbuffered 0.9% saline solution, or 5.5 buffered with sodium bicarbonate) and duration of the instillation time (1 h or 2 h) were administered as a single instillation before TUR. Three patients were to be included in each arm. Characteristics of dilution volume, pH and duration of instillation within each arm are reported in Table 1. Arm 3 proved to be impracticable because gemcitabine 40 mg/mL is not entirely soluble at a pH of 5.5.

Blood samples (5 mL) were drawn into heparinized tubes preloaded with 40 g of the cytidine deaminase inhibitor tetrahydrouridine (Calbiochem, San Diego, CA, USA) to prevent degradation of the drug. Plasma, urine and biopsy tissue samples were obtained during the post-instillation TUR from the exophytic tumor lesion, its base of implant and the normal mucosa distant from the lesion. Tissue samples were frozen and stored at −80°C until analysis. After voiding, the bladder was washed with saline solution, and cold biopsy tissue samples were obtained during the post-instillation TUR from the exophytic tumor lesion, its base of implant and the normal mucosa distant from the lesion. Tissue samples were frozen and stored at −80°C until analysis and subsequently processed according to the procedure described by Mattioli et al. [14] with minimal modifications. An aliquot of homogenized tissue was used for the Bradford protein assay [15]. Plasma, urine and tissue dFdC and dFdU were extracted from plasma, urine and tissue according to the procedure described by Yilmaz et al. [16] and analysed with a validated high performance liquid chromatography-mass spectrometry (HPLC-MS/MS), via electrospray ionization (ESI) interface as reported by Xu et al. [17] The limit of quantification (LOQ) of dFdC was 134 ng/mL and LOQ of dFdU was 5 ng/mL. Authentic gemcitabine (GEMZAR) was provided by Eli Lilly and Co. (Indianapolis, IN, USA). The dFdC was synthesized by the Department of Science and Technology of Drug, University of Turin, Italy. Internal standard 5-fluorouridine (CAS 316–46–1) was provided by Acros Organics (Geel, Belgium). Standard curves were obtained analysing, by HPLC-MS/MS, different amounts of dFdC and dFdU, as well as an internal standard, ranging between 10 ppb and 1 ppm (10 ppb, 50 ppb, 100 ppb, 125 ppb, 250 ppb, 500 ppb, 1 ppm). The mass of [dFdC–H]−, [dFdU–H]− and [5-fluorouridine–H]− was 262, 263 and 261 m/z, respectively. Data were combined and plotted to determine the correlation between the amount of dFdC, dFdU and the resulting plasma was frozen and stored at −80°C until analysis. Urine samples were collected before instillation and at voiding, the volume and pH of urine were carefully measured and recorded, and an aliquot was frozen and stored at −80°C until analysis. After voiding, the bladder was washed with saline solution, and cold biopsy tissue samples were obtained during the post-instillation TUR from the exophytic tumor lesion, its base of implant and the normal mucosa.

(1) for the ‘dilution volume’ variable, arm 1 (50 mL) vs arm 2 (100 mL) with both arms having the same pH (2.5–3.5) and instillation time (1 h);

(2) for the ‘pH’ variable, arm 2 (2.5–3.5) vs arm 4 (5.5) where both arms had the same dilution volume (100 mL) and instillation time (1 h), and arm 5 (2.5–3.5) vs arm 6 (5.5), which had the same dilution volume (100 mL) and instillation time (2 h);
(3) for the ‘instillation time’ variable, arm 2 (1 h) vs arm 5 (2 h) which had the same pH (2.5–3.5) and dilution volume (100 mL), and arm 4 (1 h) vs arm 6 (2 h) which had the same pH (5.5) and dilution volume (100 mL).

The pharmacokinetic curves (concentration vs time) and tissue concentrations of dFdC and dFdU (ng/mg of proteins) were determined using the mean value for each arm.

Results

After arm 3 had been excluded, 15 patients were enrolled in the remaining five arms between April 2007 and May 2008.

**Plasma and urine concentrations**

The highest plasmatic mean concentrations of dFdC (C\text{max} 1.44 µM) and dFdU (7.28 µM) were observed in arm 6 and arm 2 respectively, whereas the lowest C\text{max} of dFdC (0.59 µM) and dFdU (1.07 µM) in arm 1 (Figs 1 and 2). The fraction dose of dFdC usually retrieved from voided urine was always very high (> 95% of the administered dose) whereas the amount of dFdU was very low (range 0.1–2.7 mg). The mean volume and pH values of voided urine after instillation were 116 mL (100–150 mL) and 4.5 (4.0–5.0) in arm 1, 250 mL (200–300 mL) and 4.3 (3.5–5.0) in arm 2, 243 mL (150–300 mL) and 5.8 (5.5–6.0) in arm 4, 270 mL (230–350 mL) and 6.0 (5.5–6.5) in arm 5, 237 mL (200–310 mL) and 6.2 (6.0–6.5) in arm 6, respectively. The formation of urine during the instillation period lead to an increase of the final pH of voided urine, and this increase is noticeable mainly in arm 1, 2 and 5, in which the dFdC solution is unbuffered.

**Tissue concentrations**

Figure 3 shows the mean dFdC concentrations in tumor tissue, its base of implant and macroscopically normal mucosa across all treatment arms. The highest concentrations of dFdC were found in arm 6 for the tumor tissue (18267.9 ng/mg of proteins) and the normal mucosa (9979.5 ng/mg of proteins) and in arm 5 for the base of implant (6821.2 ng/mg of proteins). The lowest concentrations of dFdC for the tumor, its base of implant and normal mucosa (245.6, 268.9 and 391.9 ng/mg of proteins respectively) were observed in arm 1. The highest concentrations of dFdU in the tumor (4137.3 ng/mg of proteins) and in the base of implant (1550.0 ng/mg of proteins) were reached in arm 2, whereas for the normal mucosa (1192.8 ng/mg proteins) they were measured in arm 6. The lowest concentrations of dFdU for the tumor, its base of implant and normal mucosa (199.5, 179.7 and 260.2 ng/mg of proteins respectively) were observed in arm 1. The relationship between dFdC and dFdU tissue concentrations and the instillation volume is shown by comparing the volumes of 50 mL and 100 mL at pH 2.5–3.5 for an instillation time of 1 h. An instillation volume of 100 mL led to an increase in dFdC and dFdU concentrations of 1.5 and 21 times, respectively, in the tumor tissue, of five and nine times, respectively, in the base of implant, and of 1 and 3.5 times respectively in the normal mucosa compared with a 50 mL volume.

The influence of pH alone on dFdC and dFdU tissue concentrations could be assessed by comparing the two different pH (2.5–3.5 and 5.5) in a 100 mL solution for the 1-h and the 2-h instillation times, respectively. Following a 1-h instillation at a pH of 5.5, dFdC concentration was six times higher in the tumor tissue and five times higher in bladder mucosa compared with the same solution at a pH of 2.5–3.5, whereas dFdU concentration was several times lower at pH 5.5 than at a pH of 2.5–3.5. For the 2-h instillation, both dFdC and dFdU showed increased concentrations in the tumor tissue and normal mucosa at a pH of 5.5 compared with a pH of 2.5–3.5.

The influence of the instillation time (1 h vs 2 h) alone on dFdC and dFdU tissue concentrations was assessed in the 100 mL solution for both pH levels. At a pH of 2.5–3.5, a 2-h instillation notably increased dFdC concentration in all tissue samples (25 times in the tumor tissue, 5 times in the base of implant and 4 times in the normal mucosa) and decreased dFdU concentration in all tissue samples compared with an instillation time of 1 h. At a pH of 5.5, concentrations of both dFdC and dFdU increased after 2 h of instillation compared with an instillation time of 1 h.

Discussion

The standard dose of gemcitabine for intravesical use in intermediate and high-risk NMIBC [3–7,9,12,13], defined by phase I and II studies, is 2000 mg. However, no consensus exists on the optimal mode of administration as different dilution volumes (50 mL or 100 mL), pH levels (2.5–3.5 or 5.5) and instillation times (1 h or 2 h) have been employed in studies without any apparent pharmacokinetic rationale. Different conditions may affect systemic absorption, intracellular bioavailability and metabolism of the drug. The present study aimed to determine the plasmatic, urinary and intratissue concentrations of dFdC and dFdU in bladder cancer patients after a single instillation of gemcitabine administered before TUR, and we report for the first time the direct determination of intratissue concentrations of dFdC and dFdU after intravesical administration of the study drug under different pH conditions, dilution volumes and exposure times. Previously only the intratumoral activity of deoxycytidine kinase (the enzyme that transforms the dFdC into its active form gemcitabine triphosphate) and of deoxycytidine deaminase (the enzyme that transforms the gemcitabine into its inactive metabolite dFdU) had been assessed in an attempt to correlate these key enzymes with clinical efficacy [6]. The location of tissue
samples was selected in order to verify whether dFdC fulfills the therapeutic purposes of an intravesically administered antitumoral drug: (1) tumor ablation, assessable through the ability to enter in the tumor cells; (2) deep penetration up to the lamina propria, verifiable through the drug concentration in the base of implant; and (3) prophylaxis, that is, the ability to penetrate a macroscopically normal urothelium potentially harboring pretumoral genetic alterations [18]. dFdC plasmatic concentrations across the different time intervals analyzed proved to be remarkably low in all arms, which means that the different administration conditions had a negligible effect on the systemic absorption of the drug. The most notable finding of the study was the important changes in intratissue concentrations of dFdC and dFdU when there were different administration conditions, suggesting that these variations can heavily influence intracellular penetration and metabolism of dFdC. The highest tissue concentrations of dFdC occurred when 2000 mg gemcitabine was administered in 100 mL at a pH of 5.5 for 2 h. Under these conditions, there was a huge increase in the concentrations of dFdC inside the tumor, its base of implant and the macroscopically normal mucosa than with 2000 mg in 50 mL at a pH of 2.5–3.5 for 1 h, which is the most common administration protocol for intravesical gemcitabine reported in the literature [5,8,9,19,20]. In contrast, the increase in the intracellular dFdU concentration in the arm treated with 100 mL at pH 5.5 for 2 h was proportionally several times lower than the dFdC concentration. Comparison of intratissue concentrations between all five arms enabled assessment of the individual effect of each variable on drug absorption and metabolism. In our study an instillation volume of 100 mL, employed by some authors in clinical trials using gemcitabine [3,11,20], did not seem to increase the intracellular concentrations of dFdC as much as that of the inactive metabolite dFdU. While the former could be explained by enhanced intracellular diffusion owing to a higher solubility of the drug, no rational explanation can be provided for the latter. Increasing the pH of gemcitabine from 2.5–3.5–5.5, as adopted by Dalbagni et al. [3,11] with the intention of reducing local side-effects, was interestingly found to promote a marked intracellular accumulation of the drug in our study model. Given that it has a pKₐ of 3.6, at a pH of 5.5 gemcitabine is entirely available in its undissociated form [2] and thus more easily diffusible through tissues, unlike the counterpart at pH 2.5–3.5 where only 50% of the molecules remains undissociated. Although the standard instillation time is conventionally set at 1 h, some authors [6] have shown that holding dFdC in the bladder for 2 h is feasible and safe. Our study suggests that the drug may be more active under these conditions since its intratissue concentration is markedly increased. Notably, enhanced intracellular concentrations of dFdC following an increase in pH and a longer instillation time were accompanied by marginal increases or even decreases in intracellular concentrations of dFdU. This becomes particularly evident when comparing arms at different pH levels, where the less acid solution seems to favor low dFdU formation, probably through the inhibition of the inactivating enzyme DCTD [21]. Down-regulation of DCTD is also known to occur in the presence of high levels of difluorodeoxycytidinemonophosphate (dFdCMP), the active metabolite of gemcitabine, that might well increase linearly with the availability of dFdC into the cell [22]. The concentration dFdCMP was not determined in the present study and this should be acknowledged as a limitation of the study.

Conclusions

Changes in pH level, dilution volume and instillation time for intravesical gemcitabine result in considerable changes in tissue concentrations of dFdC and dFdU, without any significant impact on systemic absorption. The intravesical administration of gemcitabine with a solution diluted in 100 mL, at a pH of 5.5 and kept in the bladder for 2 h led to the highest intratumoral levels of dFdC with dFdU levels remaining low. This was in marked contrast with the 75 times lower dFdC tumor concentrations observed in the most commonly used intravesical gemcitabine solution (50 mL solution, at a pH of 2.5–3.5 for 1 h). Further studies are needed to determine whether more favorable pharmacokinetic properties may affect the clinical activity of gemcitabine. Finally, the proposed experimental model may be employed to optimize intravesical administration of other intravesically chemotherapy drugs.

References


Figures and Tables

**Fig. 1** Pharmacokinetic curves (mean value and SD) of plasmatic gemcitabine (2',2'-difluorodeoxycytidine, dFdC) in the different treatment arms.

**Fig. 2** Pharmacokinetic curves (mean value and SD) of plasmatic dFdU (2',2'-difluorodeoxyuridine) in the different treatment arms.
**Fig. 3** Intratissue gemcitabine, (2',2'-difluorodeoxycytidine, dFdC) and dFdU (2',2'-difluorodeoxyuridine) concentrations in the different treatment arms (mean value and SD)

![Graph showing concentrations](image)

**Table 1** Specific administration schedules (in terms of dilution volume, pH of the solution and instillation time) of the six arms receiving 2000 mg intravesical gemcitabine

<table>
<thead>
<tr>
<th>Arms</th>
<th>Dilution volumes (mL)</th>
<th>pH of solution</th>
<th>Duration of instillation (h)</th>
<th>Number of patients enrolled</th>
</tr>
</thead>
<tbody>
<tr>
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<td>50</td>
<td>pH 2.5–3.5</td>
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<td>3</td>
</tr>
<tr>
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<td>100</td>
<td>pH 2.5–3.5</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
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<td>pH 5.5</td>
<td>1</td>
<td>Arm withdrawn</td>
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<td>pH 5.5</td>
<td>1</td>
<td>3</td>
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<td>3</td>
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<tr>
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<td>3</td>
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</tbody>
</table>