New insights in pyrimidine antagonist chemotherapy
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Pharmacokinetics and pharmacogenetics of gemcitabine combined with epirubicin or cisplatin in non-small cell lung cancer patients

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Submitted
Abstract

Aim The pharmacokinetics of gemcitabine and its metabolite 2',2'-difluorodeoxyuridine (dFdU) and the influence of a common A79C polymorphism in the cytidine deaminase gene (CDD) were studied in non-small cell lung cancer patients, treated with gemcitabine plus epirubicin (EG) or gemcitabine plus cisplatin (GC).

Methods Patients were treated with gemcitabine 1125 mg/m², followed by epirubicin 100 mg/m² (EG; n=12) or cisplatin 80 mg/m² (GC; n=10). Plasma was collected during 3 h after gemcitabine administration. Gemcitabine and dFdU were quantified by High Performance Liquid Chromatography (HPLC) with ultraviolet detection. The CDD A79C genotype was determined with Polymerase Chain Reaction (PCR) and DNA sequencing.

Results Gemcitabine was rapidly cleared from plasma and undetectable after 3 h. The pharmacokinetics of gemcitabine was the same in the two treatment groups. A plasma Area Under the Curve (AUC) of 8.3 vs. 7.6 mg.h/L and a half-life of 0.20 vs. 0.16 h were calculated in respectively the EG and GC group. The dFdU half-life was larger in EG compared to GC treated patients (3.3 vs. 1.2 h; p<0.05). The influence of the CDD A79C genotype was studied in 20 patients. There was a trend towards higher AUC (+16%; p=0.20) and lower clearance values (-11%; p=0.35) in individuals heterozygous or homozygous for the C-genotype (C/C; A/C; n=12), compared to homozygotes for the A-genotype (A/A; n=8).

Conclusion The pharmacokinetics of gemcitabine is similar in EG treated compared to GC treated patients. The influence of a common A79C polymorphism in the CDD gene on gemcitabine pharmacokinetics is probably only minor.

Introduction

During the last decade, several new chemotherapeutic agents have been evaluated for their beneficial effect in patients with non-small cell lung cancer (NSCLC). So far, gemcitabine plus cisplatin has emerged as one of the standard regimens for the treatment of advanced NSCLC [1]. Unfortunately, cisplatin can cause nephro-, neuro-, and ototoxicity. Moreover, cisplatin administration requires pre- and post hydration. Thus, in search for a better tolerable, easy to administer, non-platinum regimen, the activity of gemcitabine combined with epirubicin (EG) was studied in our hospital in patients with advanced NSCLC [2]. Main toxicities of the EG combination, observed during phase I/II trials were granulocytopenia, thrombocytopenia, febrile neutropenia and mucositis.

Only few reports are available with respect to the pharmacokinetic interaction between gemcitabine and epirubicin. Perez-Manga et al. studied the pharmacokinetics of gemcitabine 800 mg/m² in the presence of a low dose (25 mg/m²) doxorubicin and found that the tissue disposition and clearance of both drugs was unchanged [3]. Fogli et al. studied the pharmacokinetics of 90 mg/m² epirubicin in the presence of 1000 mg/m² gemcitab-
Gemcitabine pharmacokinetics and pharmacogenetics. They concluded that gemcitabine has no effect on epirubicin pharmacokinetics [4]. However, the effect of epirubicin on the gemcitabine pharmacokinetics was not studied. The clearance of gemcitabine is primarily determined by its degradation into 2',2'-difluorodeoxyuridine (dFdU) by the enzyme cytidine deaminase [5]. Cytidine deaminase is ubiquitous in the human body, catalyzing the hydrolytic deamination of (deoxy-)cytidine to (deoxy-)uridine. The total enzyme capacity of cytidine deaminase in all organs and tissues determines the biotransformation rate of gemcitabine into dFdU and thereby indirectly the duration of exposure to gemcitabine. Interestingly, cloning of human cytidine deaminase has revealed a A79C polymorphism in exon 1 of the CDD gene corresponding with two protein variants (CDD1 and CDD2) with more than 2-fold difference in vitro deamination rates [6]. The C genotype corresponds to a Gln carrying enzyme (CDD-1) and the A genotype to a Lys-carrying variant (CDD-2). The CDD-2 enzyme exerts a 1.3 - 3.3 fold higher deamination rate of cytarabine than CDD-1 [7]. Thus, apart from a possible interaction with epirubicin, the pharmacokinetics of gemcitabine may be affected by this genetic polymorphism.

The aim of the current study was to determine the pharmacokinetics of gemcitabine in EG compared to GC treated NSCLC patients. Information on pharmacokinetic and pharmacodynamic interactions between anti-cancer drugs is essential for optimizing the efficacy-toxicity ratio of a treatment schedule. Additionally, the effect of the A79C polymorphism in the cytidine deaminase gene in relation to gemcitabine pharmacokinetics was explored.

**Patients and methods**

**Patient selection**
This study was performed as a site study of a phase III trial of gemcitabine plus epirubicin versus gemcitabine plus cisplatin (GC) in advanced NSCLC patients [8]. Patients had to meet the inclusion and exclusion criteria of the main study. In short, patients were included if they had stage IIIB/IV NSCLC. No prior chemotherapy was allowed. An adequate bone marrow reserve, normal renal and liver function were required. The glomerular filtration rate (GFR) was calculated according to the formula of Cockcroft and Gault [9]. Patients were excluded if they had active infections, second primary malignancies, uncorrected hypercalcaemia or a left ventricular ejection fraction (LVEF) less than 45%. A detailed description of the inclusion and exclusion criteria is published elsewhere [8]. The local medical ethics committee of the hospital approved the protocol. All patients gave informed consent before study entry.
Treatment and sample collection

Gemcitabine-Epirubicin group

Gemcitabine (Gemzar®, Lilly, Nieuwegein, The Netherlands) in a dose of 1125 mg/m² in 250 mL 0.9% NaCl solution was administered as a 30 min infusion on day 1 and day 8. Epirubicin (Farmorubicine®, Pharmacia, Woerden, The Netherlands) in a dose of 100 mg/m² in 50 mL 0.9% NaCl solution was administered as an intravenous bolus injection over 5 min, immediately after the end of the gemcitabine infusion, on day 1 of each 21-day cycle. For pharmacokinetic sampling, a cannula was placed intravenously in the arm of the patient contralateral to the side of drug administration. Blood samples of 9 mL were collected in heparinized tubes containing 0.25 mg tetrahydrouridine (THU) in 50 µL water, just before chemotherapy and at t = 25, 40, 50, 60, 75, 90, 105, 120, 150, 180, 270, 360, 540 and 1320 min after start of the gemcitabine infusion.

Gemcitabine-Cisplatin group

Gemcitabine (Gemzar, Lilly, Nieuwegein, The Netherlands) in a dose of 1125 mg/m² in 100 mL 0.9% NaCl solution was administered as a 30 min infusion on day 1 and day 8. Cisplatin in a dose of 80 mg/m² in 1000 mL 0.9% NaCl solution was administered as a 3 h infusion, starting 1 h after the end of the gemcitabine infusion, on day 1 of each 21-day cycle. Blood samples of 9 mL were collected in heparinized tubes containing 0.25 mg tetrahydrouridine (THU) in 50 µL water, just before chemotherapy and at t = 25, 40, 50, 60, 75, 90, 105, 120, 150, 180, and 240 min after start of the gemcitabine infusion. Plasma and saliva samples were kept frozen at - 20 ºC until analysis.

Pharmacokinetic analysis

Gemcitabine hydrochloride, dFdU, 2’‘,2’‘-difluorodeoxycytidine monophosphate (dFdCMP), 2’‘,2’‘-difluorodeoxycytidine diphosphate (dFdCDP), 2’‘,2’‘-difluorodeoxycytidine diphosphate (dFdCTP) were obtained from Lilly Co., (Indianapolis, IN). Tetrahydrouridine was purchased from Calbiochem (La Jolla, CA). All other chemicals were of standard analytical grade.

The analysis of gemcitabine and dFdU in plasma was carried out as described by Freeman et al. [10].

Cytidine deaminase genotyping

The CDA exon 1 polymorphism (C/A) at codon position 27 was genotyped by direct sequencing, in both directions, of PCR amplified genomic DNA.

First, the CDA region flanking the polymorphic site was amplified using PCR with forward primer 5’- AGTAGCTTCCCCTCCAGTAGC and reversed primer 5’- CCTCTTCTTGACATCCTTCCCT. The 25 µL reactions contained: 2.5 units Taq polymerase (Amersham Biosciences, Uppsala, Sweden); 0.5 mM dNTP mix (Roche Diagnostics, Mannheim, Germany); 1x PCR buffer (Roche Diagnostics), 0.05 mM MgCl₂; 0.2 µM of each primer; and approximately
50 ng genomic DNA. The amplification was performed on a PTC-225 thermal cycler (MJ Research, Waltham, MA), using a stepdown protocol. The first 5 cycles were carried out at 94 °C, 65 °C, and 72 °C, each for 30 s. The next 5 cycles at 94 °C, 63 °C, and 72 °C, each for 30 s. The last 25 cycles at 94 °C, 60 °C, and 72 °C, each for 30 s. Following cycling the PCR products were purified with the Qiagen Qiaquick PCR purification kit (Westburg b.v, Leusden, the Netherlands). Subsequently, 100 ng of the purified PCR product was cycle sequenced with a Dyeterminator kit (US81090, Amersham Biosciences, Roosendaal, the Netherlands) in a thermal cycler (MJ Research), using 0.05 mM sequencing primer. For the reverse reaction the same primer was used as in the PCR, but for the forward reaction an internal primer was used (5'-GGTACCAACATGGCCCAGAAG). After the cycle reaction the sequencing products were cleaned on a Sephadex plate (Amersham Biosciences) by centrifugation for 5 min at 910 g. The eluted sample was vacuum dried for 45 min at 65 °C. Finally, 20 µL of loading solution (Amersham Biosciences) was added to dissolve the sequencing products. The samples were analyzed on a MegaBACE 1000 capillary sequencer (Amersham Biosciences) by injecting the samples for 45 s at 3 kV, and running them for 5 hours at 4 kV. The data were processed with Sequence Analyser 3.0 (Amersham Biosciences) and Seqman II (DNASTAR Inc., Madison, WI).

**Pharmacokinetic modelling**
Pharmacokinetic data were analyzed in MwPharm (version 3.5; MediWare, Groningen, The Netherlands) using KinFit. For both gemcitabine and epirubicin and their metabolites, the AUC (trapezoid rule) and elimination half-lifes were calculated by non-compartmental analysis. Parent drug-metabolite pharmacokinetic relations were analyzed by multi-compartment analysis in the ADAPT II Maximum Likelihood Parameter Estimation program (version 4.0; University of Southern California, Los Angeles, CA). Variance for the observations was assumed to be proportional to the measured values and set at 10%. The Akaike Information Criterion (AIC) was used for model selection. The model with the lowest summarized AIC value was selected as the better one.

**Statistical analysis**
Patient data were analyzed in two groups, based on treatment schedule. Clinical chemistry and pharmacokinetic data were compared with a two sided Student’s t-test. In case of unequal variances, as indicated by the Kolmogorov-Smirnov test, data were tested with the non-parametric Mann-Whitney U-test. The study was powered (>80%) to detect a 30 % difference in population means, assuming a standard deviation in pharmacokinetic parameters of 30%. Correlations between clinical chemistry, demographic, pharmacogenetic and pharmacokinetic data were tested by Spearman Rank correlation analysis. Statistical significance was at p<0.05. Analyses were performed with the SYSTAT 7.0 statistical package (SPSS inc. 1997).
Results

Patients
Between November 2000 and September 2002 we included 12 patients in the EG group and 10 patients in the CG group. Patient characteristics of both groups are represented in table 1. Mean serum creatinine level in EG treated patients was $86 \pm 15 \text{ µmol/L}$ (mean ± s.d) and higher compared to $72 \pm 13 \text{ µmol/L}$ in GC treated patients. The GFR revealed a lower rate of $74 \pm 17 \text{ mL/min}$ in EG compared to $93 \pm 21 \text{ mL/min}$ in GC treated patients (p<0.02). No differences were observed in other parameters.

Pharmacokinetics
Mean pharmacokinetic curves of gemcitabine and dFdU in plasma are represented in figure 1 for both treatment groups. Gemcitabine was rapidly cleared from plasma and undetectable after 3 h in all patients. Both gemcitabine and dFdU data were fitted in a parent drug – metabolite model, comprising one compartment for gemcitabine and two compartments for dFdU pharmacokinetics. The pharmacokinetic data of the two groups is represented in table 2. No differences were measured in the pharmacokinetics of gemcitabine. However, the half-life of dFdU appeared larger in EG compared to GC treated patients. This also resulted in a larger AUC of dFdU in the EG group. In a Spearman rank correlation analysis, only a weak correlation was found between dFdU plasma half-life and serum creatinine (R=0.39) or GFR (R=0.40) (see figure 2).

Table 1  Patient characteristics in both treatment groups.

<table>
<thead>
<tr>
<th></th>
<th>EG (n=12)</th>
<th>GC (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>10/2</td>
<td>11/1</td>
</tr>
<tr>
<td>Age (y)</td>
<td>$63 \pm 9$</td>
<td>$65 \pm 7$</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>$77 \pm 13$</td>
<td>$77 \pm 13$</td>
</tr>
<tr>
<td>Length (cm)</td>
<td>$178 \pm 11$</td>
<td>$176 \pm 4$</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>$1.95 \pm 0.21$</td>
<td>$1.92 \pm 0.15$</td>
</tr>
<tr>
<td>Serum-Creatinine (µmol/L)</td>
<td>$86 \pm 15$</td>
<td>$72 \pm 13 ^*$</td>
</tr>
<tr>
<td>GFR (mL/min)</td>
<td>$74 \pm 17$</td>
<td>$93 \pm 21 ^*$</td>
</tr>
<tr>
<td>Serum-AST (U/L)</td>
<td>$23 \pm 4$</td>
<td>$21 \pm 6$</td>
</tr>
<tr>
<td>Serum-ALT (U/L)</td>
<td>$35 \pm 7$</td>
<td>$27 \pm 8$</td>
</tr>
<tr>
<td>Serum-LDH (U/L)</td>
<td>$101 \pm 52$</td>
<td>$110 \pm 47$</td>
</tr>
<tr>
<td>Serum-ALP (U/L)</td>
<td>$239 \pm 88$</td>
<td>$234 \pm 59$</td>
</tr>
<tr>
<td>Serum-Bilirubin (µmol/L)</td>
<td>$10 \pm 2$</td>
<td>$12 \pm 3$</td>
</tr>
</tbody>
</table>

Mean values ± the standard deviation are presented; ^* different at p<0.05 (Student’s t-test)
Table 2  Gemcitabine pharmacokinetic parameters in both treatment groups.

<table>
<thead>
<tr>
<th></th>
<th>EG (n=12)</th>
<th>GC (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemcitabine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose (mg)</td>
<td>2136 ± 173</td>
<td>2180 ± 274</td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0→3 h&lt;/sub&gt; (mg.h/L)</td>
<td>8.3 ± 2.9</td>
<td>7.6 ± 1.5</td>
</tr>
<tr>
<td>V&lt;sub&gt;distribution&lt;/sub&gt; (L)</td>
<td>60 ± 20</td>
<td>63 ± 20</td>
</tr>
<tr>
<td>Cl&lt;sub&gt;metabolic&lt;/sub&gt; (L/h)</td>
<td>240 ± 147</td>
<td>298 ± 66</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2 elimination&lt;/sub&gt; (h)</td>
<td>0.20 ± 0.08</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>dFdU</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC&lt;sub&gt;0→3 h&lt;/sub&gt; (mg.h/L)</td>
<td>67 ± 14</td>
<td>56 ± 8&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;1/2 elimination&lt;/sub&gt; (h)</td>
<td>3.3 ± 1.4</td>
<td>1.2 ± 0.2&lt;sup&gt;‡&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Mean values ± the standard deviation are presented<sup>‡</sup> different at p<0.05 (Student’s t-test)
Gemcitabine pharmacokinetics and pharmacogenetics

Pharmacogenetic analysis
The pooled gemcitabine pharmacokinetic data of all 22 patients were analyzed with respect to the influence of the A79C polymorphism in the CDD gene. These data are represented in table 3. The mean pharmacokinetic curves of gemcitabine and dFdU according to CDD genotype are represented in figure 3.

The A genotype corresponds to the CDD-2 variant of cytidine deaminase, which has a 2-fold higher intrinsic activity than the to the C genotype corresponding CDD-1 variant. Our data indeed show a trend towards higher AUC (+16%; p=0.20) and lower clearance values (-11%; p=0.35) in individuals heterozygous or homozygous for the C-genotype (C/C; A/C; n=12), compared to homozygotes for the A-genotype (A/A; n=8).

Table 3  Gemcitabine drug elimination related parameters, as measured in each subgroup according to cytidine deaminase A79C genotype.

<table>
<thead>
<tr>
<th></th>
<th>A/A (n=8)</th>
<th>C/C (n=4)</th>
<th>A/C (n=8)</th>
<th>A allele bearing (n=16)</th>
<th>C allele bearing (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC (mg.h/L)</td>
<td>8.00 ± 1.56</td>
<td>9.79 ± 3.01</td>
<td>9.31 ± 3.31</td>
<td>8.75 ± 2.70</td>
<td>9.47 ± 3.08</td>
</tr>
<tr>
<td>Cl (L/h)</td>
<td>271 ± 50</td>
<td>245 ± 126</td>
<td>239 ± 60</td>
<td>252 ± 56</td>
<td>241 ± 82</td>
</tr>
<tr>
<td>T½ elimination (h)</td>
<td>0.19 ± 0.06</td>
<td>0.20 ± 0.05</td>
<td>0.18 ± 0.06</td>
<td>0.18 ± 0.06</td>
<td>0.18 ± 0.06</td>
</tr>
</tbody>
</table>

Mean values ± the standard deviation are presented.
In this study, we investigated the effect of epirubicin on the pharmacokinetics of gemcitabine in NSCLC patients treated with both drugs. In all patients gemcitabine was rapidly cleared from plasma, as was expected from previous reports [11]. We observed no differences in gemcitabine clearance between EG and GC treated patients, indicating lack of interaction between gemcitabine and epirubicin. This is in line with previous findings of Conte et al. who reported that the pharmacokinetics of gemcitabine remains unchanged in the presence of 175 mg/m\(^2\) paclitaxel and 90 mg/m\(^2\) epirubicin [12]. It was known already that cisplatin does not alter gemcitabine pharmacokinetics [13]. Interestingly, the clearance of the gemcitabine metabolite dFdU was lower in EG compared to GC treated patients. Since dFdU elimination is mainly determined by renal excretion we analyzed the effect of renal function. Although the renal function was about 20% better in GC compared to EG treated patients, we only found a weak correlation between dFdU clearance and serum creatinine or glomerular filtration rate. This suggests that, apart from renal function, also another factor contributes to the observed difference. We hypothesized that the shorter half-life of dFdU in GC treated patients may in part be the result of increased dFdU excretion and/or tissue disposition due to pre-hydration measures.

Figure 3  Pharmacokinetics of gemcitabine and its metabolite dFdU after a 30 min infusion of 1125 mg/m\(^2\) gemcitabine grouped according to genotype of the cytidine deaminase A79C polymorphism. Error bars represent the s.d. values.
necessary for cisplatin administration. Enhanced elimination due to hydration is known to occur for a few drugs, including the anticancer agent methotrexate [14,15]. Another possibility could be an interaction between dFdU and cisplatin or epirubicin. Interestingly, increased elimination of dFdU has also been reported by Van Moorsel et al. when they administered gemcitabine 4 hours after cisplatin compared to 4 h before cisplatin [13]. In our study, the cisplatin administration was started 1 h after the end of the gemcitabine infusion in GC treated patients. Thus, dFdU pharmacokinetics may have been influenced by the presence of cisplatin. Since dFdU is considered to be biologically inert, the observed difference was considered of no clinical importance [16].

The most important factor that determines the clearance rate of gemcitabine is the activity of the enzyme cytidine deaminase. This enzyme rapidly catabolizes gemcitabine. The enzyme capacity of cytidine deaminase in all organs and tissues determines the biotransformation rate of gemcitabine into dFdU and thus the duration of exposure to gemcitabine. Therefore, we determined whether gemcitabine metabolism was affected by a known A79C polymorphism in exon 1 of the CDD encoding gene. The C genotype corresponds to the Gln carrying enzyme (CDD-1) and the A genotype to the Lys-carrying natural variant (CDD-2). The CDD-2 enzyme has been shown to exert a 1.3 - 3.3 fold higher deamination rate of cytarabine than CDD-1 [7]. The frequencies of the A/A, A/C and C/C genotype were 42%, 37% and 21% respectively in our study. We observed no large differences in gemcitabine clearance between the different genotypes. Our data showed a trend towards higher AUC and lower clearance values in individuals who are heterozygous or homozygous for the C-genotype, compared to homozygotes for the A-genotype. These differences however did not reach statistical significance. The observed difference in clearance was 11% and in AUC 16%, while the standard deviation reached nearly 33%. Based on these data, we calculated that at least 120 patients are required to proof the influence of the A79C polymorphism on gemcitabine pharmacokinetics.

Interestingly, recently two new polymorphisms in the CDD gene have been reported. These concern the G208A and T435C polymorphism with allele frequencies of 4.3% and 70.1% respectively [17]. The G208A polymorphism produces a alanine to threonine substitution (A70T) within the conserved catalytic domain. Introduction of this gene in yeast null mutants, resulted in a 20% reduction of the 50% inhibitory concentration value for cytarabine [17]. The impact of this polymorphism on gemcitabine clearance is unclear.

The presence of several polymorphisms in the cytidine deaminase gene, with at least two affecting the catalytic domain, may partly explain the interindividual variability in enzyme activity. Since two polymorphisms already result in nine different genotypes, the full pharmacogenetic picture of cytidine deaminase in relation to gemcitabine clearance may be quite complex and can only be elucidated in larger clinical trials.

We conclude that the pharmacokinetics of gemcitabine is similar in EG treated compared to GC treated patient. The influence of a common A79C polymorphism in the cytidine deaminase gene on gemcitabine pharmacokinetics is probably only minor.
References


