Relationship between exposure and toxicity in high-dose chemotherapy with cyclophosphamide, thiotepa and carboplatin

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Background: High-dose chemotherapy in combination with peripheral blood progenitor cell transplantation is widely used in the treatment of several malignancies. The use of high-dose chemotherapy can be complicated by the occurrence of severe and sometimes life threatening toxicity. A wide inter-patient variability in toxicity is encountered, which may be caused by variability in the pharmacokinetics of the agents. The aim of this study was to establish the pharmacokinetics of cyclophosphamide, thiotepa, carboplatin and all relevant metabolites in a widely used high-dose combination and to study possible relationships between the pharmacokinetics and toxicity.

Patients and methods: Blood samples were collected from patients treated with modifications of the CTCb regimen consisting of cyclophosphamide (1000–1500 mg/m²/day), carboplatin (265–400 mg/m²/day) and thiotepa (80–120 mg/m²/day) as short infusions for four consecutive days. Thiotepa and its main metabolite tepa, ultrafilterable carboplatin, cyclophosphamide and its activated metabolites 4-hydroxycyclophosphamide and phosphoramide mustard were determined. Pharmacokinetics were assessed with the use of population pharmacokinetic analyses. Relationship between the area under the concentration–time curves (AUCs) of these compounds and toxicity were tested.

Results: A total of 46 patients (83 courses of chemotherapy) was included. Relationships were identified between elevation of transaminases and the thiotepa and tepa AUC, mucositis and the tepa AUC and ototoxicity and the carboplatin AUC. A strong trend between the 4-hydroxycyclophosphamide AUC and veno-occlusive disease was found.

Conclusions: The complex pharmacokinetics of the different agents and their metabolites have been established and several relationships between the pharmacokinetics and toxicity were identified. These findings may form the basis for further treatment optimisation and dose-individualisation in this high-dose chemotherapy combination.

Key words: carboplatin, cyclophosphamide, high-dose chemotherapy, pharmacokinetics, thiotepa, toxicity

Introduction

High-dose chemotherapy in combination with peripheral blood progenitor cell (PBPC) or bone marrow transplantation is widely used in the treatment of several haematological malignancies and solid tumours. The doses of commonly used antineoplastic agents have been increased substantially in these high-dose regimens compared with regimens that are given without stem cell support [1, 2]. High-dose chemotherapy was widely viewed as an effective treatment modality in breast cancer. However, no positive randomised studies with sufficient follow-up have been reported thus far. Several years of data maturation and the results of additional randomised trials must be awaited [3–5].

One particularly well-established regimen in high-dose chemotherapy for solid tumours is the CTCb regimen which consist of a combination of cyclophosphamide (6000 mg/m²), thiotepa (500 mg/m²) and carboplatin (800 mg/m²) all given as continuous 96 h infusions [3, 6]. Two related regimens, commonly used in The Netherlands, are the CTC regimen, which

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consists of cyclophosphamide (6000 mg/m²), thiotepa (480 mg/m²) and carboplatin (1600 mg/m²) divided over 4 days administered as short infusions and the tCTC regimen which is identical to the CTC regimen except that the doses are reduced to two-thirds [7–9]. The dose of carboplatin used in these regimens was considerably higher than in the original CTCb regimen. Furthermore, the development of the tCTC regimen allowed the administration of multiple courses of high-dose chemotherapy.

The use of these high-dose combinations can be complicated by the occurrence of severe and sometimes life-threatening toxicities such as severe mucositis, haemorrhagic cystitis, renal failure, veno-occlusive disease (VOD), cardiac toxicity, hearing loss and sensory neuropathy [3, 8–10]. A wide interpatient variability in toxicity is encountered and insight into factors contributing to this variability may form the basis for further treatment optimisation. The pharmacokinetics of the agents included in the regimen and their metabolites may be one of these factors.

The pharmacokinetics and pharmacodynamics of commonly used agents in high-dose chemotherapy have been reviewed recently [1]. Cyclophosphamide and thiotepa are both extensively metabolised. Cyclophosphamide, a prodrug, is activated by cytochrome P450 to 4-hydroxycyclophosphamide, which leads to decomposition into the final cytotoxic metabolite phosphoramide mustard. In addition to this activation route, cyclophosphamide is excreted unchanged in the urine and is deactivated to 2-dechloroethylcyclophosphamide. Thiotepa is metabolised by cytochrome P450 to tepa, which shows comparable alkylating activity to thiotepa [11]. Recently, we have identified two other metabolites of thiotepa, thiotepa-mercapturate and monochlorotepa, in the urine of patients treated with thiotepa [12]. Thiotepa markedly inhibits the conversion of cyclophosphamide to 4-hydroxycyclophosphamide by inhibition of the cytochrome P450 iso-enzyme involved [13]. This interaction leads to a lower cyclophosphamide clearance in this combination and to a decreased bioactivation, but the clinical consequences remain unclear [13]. Carboplatin binds irreversibly to plasma proteins and the free, ultrafilterable fraction is considered pharmacologically active.

Some relationships between the pharmacokinetics of cyclophosphamide, thiotepa and carboplatin and toxicity in high-dose chemotherapy have been described. For cyclophosphamide, cardiotoxicity has been shown to be inversely related to the cyclophosphamide area under the concentration–time curve (AUC) [14, 15], which could be explained by an inverse relationship between cyclophosphamide AUC and the bioactivation of cyclophosphamide. The occurrence of mucositis and severe regimen-related toxicity has been related to the exposure to both thiotepa and tepa [16, 17]. Furthermore, ototoxicity after high-dose carboplatin has been related to the cumulative exposure to carboplatin [18]. Such relationships between exposure and toxicity can form the basis for pharmacokinetic-guided dosing strategies, which may prevent excessive toxicity. Most studies, however, have focussed on the pharmacokinetics of one compound in relation to the toxicity after a combination regimen, while it is likely that toxicity may be the result of the combination of antineoplastic agents and their metabolites.

The aim of this study was, therefore, to establish the pharmacokinetics of cyclophosphamide, thiotepa, carboplatin and all relevant metabolites in a typical high-dose combination and to study possible relationships between the pharmacokinetics and toxicity.

**Patients and methods**

**Patients**

Patients were subjected to high-dose chemotherapy protocols with PBPC transplantation. Patients had either high-risk primary breast cancer and received high-dose chemotherapy as part of their adjuvant treatment, or advanced breast, germ-cell or ovarian cancer. All patients were <60 years of age with a good performance status (WHO 0 or 1) [8, 9]. For patients with breast cancer, no previous chemotherapy was allowed, unless it had been limited to non-anthracycin-based adjuvant therapy at least 1 year before relapse. Patients with germ-cell cancer and ovarian cancer had received prior chemotherapy, which usually included cisplatin. Before the first course, patients had to have adequate renal function (creatinine clearance >60 ml/min) and hepatic function (bilirubin <20 µmol/l, ALAT and ASAT <1.5-fold × the upper limit of normal). All patients received induction and PBPC mobilisation chemotherapy followed by G-CSF (filgrastim) prior to high-dose chemotherapy as previously described in detail [8, 9].

Two different high-dose schedules, both based on CTC, were administered. The full-dose CTC regimen consisted of cyclophosphamide 1500 mg/m² as a daily 1 h infusion, 400 mg/m² carboplatin as a daily 1 h infusion and 60 mg/m² thiotepa as twice daily 30 min infusions during four consecutive days. The second regimen was the ‘tiny’ CTC regimen (tCTC), which was identical to the CTC regimen except that it incorporated precisely two-thirds of the dose of each agent. Patients received either one (high-risk primary breast cancer) or two (refractory germ-cell cancer) courses of full-dose CTC or two (metastatic ovarian cancer) or three (metastatic breast or germ-cell cancer) of tCTC, when possible every 4 weeks. Treatment delays and dose adaptations were executed as described previously [8, 9]. Before a second or third course of tCTC was started, creatinine clearance had to exceed 40 ml/min and renal function loss as determined by the 24 h creatinine clearance had to be <20% of the baseline estimate. Furthermore, the hepatic function had to have recovered (bilirubin <20 µmol/l, ALAT and ASAT <2-fold × the upper limit of normal).

MESNA (500 mg) was administered six times daily for a total of 36 doses, beginning 1 h prior to the first cyclophosphamide infusion. All patients received anti-emetics both prophylactically and as indicated, which always included dexamethasone and granisetron. Patients received prophylactic antibiotics, including ciprofloxacin and amphotericin B orally, starting 4 days before chemotherapy. Approximately 60 h after the last thiotepa infusion the PBPC were reinfused.

This study and all protocols were approved by the Committee on the Medical Ethics of the Netherlands Cancer Institute and written informed consent was obtained from all patients.
Toxicity

The toxicity of the high-dose courses was assessed during and after each course. Toxicity was graded with the National Cancer Institute Common Toxicity Criteria (NCI-CTC) [19]. The clinical diagnosis of VOD was based on elevation of transaminases, hepatomegaly and ascites. Some infrequently occurring toxicities (e.g. ototoxicity and neuropathy) were scored with the NCI-CTC and subsequently transformed to dichotomous variables (e.g. no toxicity versus toxicity of any grade).

Sampling and analysis

Blood samples were collected from a double lumen Hickman or a normal double lumen catheter inserted in a subclavian vein. The lumen ending most downstream was used for administration of the drugs, the proximal lumen was used for the collection of blood samples [20].

According to the planned sampling scheme, samples were collected prior to the start of infusions on all days of chemotherapy, and on day 1 and on days 2, 3, or 4, at 30 min after the start of the cyclophosphamide infusion (t=30) and at t=60 (end of cyclophosphamide infusion), 90, 120 (end of carboplatin infusion), 150 (end of thiotepa infusion), 165, 180, 210, 285, 390 and 660 min. On day 5 an additional sample was collected 22 h after the last cyclophosphamide infusion.

Samples were immediately placed on ice, plasma was separated by centrifuging the sample at 3000 g for 3 min at 4°C. A 1.0 ml volume of plasma was immediately added to 100 µl of a 2 M semicarbazide solution for the determination of 4-hydroxycyclophosphamide. Another 1.0 ml was added to 100 µl of a 2 M semicarbazide with 4 M sodium chloride for the determination of phosphoramidate mustard. For the determination of free carboplatin, plasma ultrafiltrate was prepared with the Amicon micropartition system with a YMT-14 membrane (Millipore; Bedford, MA, USA). Ultrafiltrate was prepared by transferring 1.0 ml plasma in the micropartition system and centrifuging the system at 1500 g for 10 min. The remaining plasma layer was collected and all samples were stored at –70°C until analysis.

Thiotepa, tepa and cyclophosphamide were quantitated with a validated gas chromatographic assay after liquid–liquid extraction with chloroform. Accuracy, within-day and between-day precision were below 10% for this assay with a lower limit of quantitation (LLQ) of 5 ng/ml (0.026 µM) for thiotepa, 5 ng/ml (0.029 µM) for tepa and 50 ng/ml (0.19 µM) for cyclophosphamide [21]. 4-Hydroxycyclophosphamide was determined as a semicarbazone derivative with a previously described and validated high-performance liquid chromatography (HPLC) assay. Accuracy, within-day and between-day precision were <7% with a LLQ of 50 ng/ml (0.18 µM) [22]. Phosphoramidate mustard was quantitated with a validated and previously described HPLC assay after stabilisation of 4-hydroxycyclophosphamide and derivatisation of phosphoramidate mustard with diethyldithiocarbamate. Accuracy, within-day and between-day precision were below 11% with a LLQ of 50 ng/ml (0.23 µM) [23]. Carboplatin was determined in plasma ultrafiltrate with a Zeeman atomic absorption spectrometry assay as described previously. The LLQ was 0.24 µM with an accuracy, within-day and between-day precision of <10% [24].

Pharmacokinetics

For all drugs and metabolites, population pharmacokinetic analyses were performed with the non-linear mixed effect modelling program NONMEM (double precision, version V1.1) [25]. These population analyses enabled the estimation of the pharmacokinetic parameters in the population (fixed effects), interindividual variability (IIV), interoccasion (course-to-course) variability (IOV) and residual variability (random effects). IIV and IOV were estimated with a proportional error model as suggested by Karlsson and Sheiner [26]. Residual variability was estimated for all compounds with a combined proportional and additive error model. Individual empirical Bayesian estimates for all pharmacokinetic parameters were obtained with the POSTHOC option of NONMEM [25]. The first-order estimation method was used for all analyses.

Figure 1 shows a graphical representation of the pharmacokinetic models used. A combined pharmacokinetic model was developed for thiotepa and tepa. The pharmacokinetics of both compounds were described with a two-compartmental model. Full details of this model have been described previously [27]. For carboplatin, an open two-compartmental model with first-order elimination from the central compartment was used as described previously [28]. For cyclophosphamide and its activated metabolites pharmacokinetic models have been developed as described previously [29]. Briefly, for cyclophosphamide and 4-hydroxycyclophosphamide an integrated pharmacokinetic model has been developed which includes the effects of auto-induction and the interaction with thiotepa. Clearance of cyclophosphamide was described by an non-inducible route (CL_h) and an inducible route (CL_ia) leading to 4-hydroxycyclophosphamide. The latter route was mediated by a hypothetical amount of enzyme. Auto-induction led to a zero-order increase in the amount of enzyme during treatment with a rate constant of k_h. Inhibition by thiotepa was modelled as a reversible, non-competitive, concentration-dependent deactivation reaction described by an association constant (K_h) and dissociation constant (k_ha). For modelling of the interaction, the empirical Bayesian estimates of the thiotepa population analysis were used.

Phosphoramidate mustard pharmacokinetics were described with a separate model with the use of the empirical Bayesian estimates of the cyclophosphamide/4-hydroxycyclophosphamide model. Phosphoramidate mustard data were described by first-order formation (k_{hp-mp}) and first-order elimination (k_{pm}). For thiotepa, tepa and carboplatin, the AUC was calculated from the estimated pharmacokinetic parameters (clearance, fraction converted to metabolite and elimination rate constant of metabolite). For cyclophosphamide, 4-hydroxycyclophosphamide and phosphoramidate mustard, the AUC was calculated using ‘dummy’ compartments of which the mass transport per unit time was equal to the concentration at that time. The amount in this compartment at time infinity was equal to the AUC of these compounds.

Statistical analysis

The AUC values of the different compounds were related to the toxicity. Besides these AUCs, the sum of the thiotepa and tepa AUC was tested since both compounds show comparable activity. Several methods were used to study the relationship between pharmacokinetics and toxicity. Relationships between toxicity after the first course and the pharmacokinetics of the first course were tested with the Kruskal–Wallis test for the toxicities scored with the NCI-CTC. For toxicity scored on a dichotomous scale logistic regression was used.

Severe toxic events mainly occur after several courses. Therefore, relationships between the cumulative AUC and these toxicities were tested with logistic regression. In order to assess whether the AUC of the first course is a prognostic factor for toxicity after subsequent courses, logistic regression for severe organ toxicity was used with both the pharmacokinetics of the first course and the number of courses administered as co-variates. Other possible confounding variables (e.g. cisplatin pretreatment) were taken into account by including these variables as co-variates in the logistic regression models.
Significance of the logistic regression models was assessed with the Wald statistic. When several significant (possibly correlated) co-variates were identified in the logistic regression analyses, step-wise forward regression with removal testing based on the probability of the Wald statistic was used. The Statistical Service Solution for Windows version 10 (SPSS; Chicago, IL, USA) was used and a significance level of 0.05 was used for all tests.

Results

A total number of 46 (male/female: 5/41) patients who received 83 courses of high-dose chemotherapy were included.

Table 1. Patients and treatment

<table>
<thead>
<tr>
<th>Disease</th>
<th>Regimen</th>
<th>No. of patients</th>
<th>No. of courses</th>
<th>No. of first courses</th>
<th>No. of second courses</th>
<th>No. of third courses</th>
</tr>
</thead>
<tbody>
<tr>
<td>High risk primary breast cancer</td>
<td>1 CTC</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastatic breast cancer</td>
<td>3 tCTC</td>
<td>16</td>
<td>44</td>
<td>16</td>
<td>16</td>
<td>12†</td>
</tr>
<tr>
<td>Refractory germ-cell cancer</td>
<td>2 CTC</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Refractory germ-cell cancer</td>
<td>3 tCTC</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Metastatic ovarian cancer</td>
<td>2 tCTC</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>46</td>
<td>83</td>
<td>46</td>
<td>23</td>
<td>14</td>
</tr>
</tbody>
</table>

*Two-third courses of tCTC were administered without cyclophosphamide due to haemorraghic cystitis after the second courses, one-third course administered with all doses reduced to 75%.

In Table 1, the details of the patient population and treatments are summarised. All patients with germ-cell cancer and one of the five patients with ovarian cancer were pretreated with cisplatin, the other four ovarian cancer patients received carboplatin as first-line chemotherapy. The median time between multiple courses of tCTC (calculated from the day of reinfusion) was 21 days (range 20–28). For the two patients who received two courses of CTC, the time between the two courses was 33 and 35 days, respectively. The median length of hospitalisation (calculated from the day of reinfusion) was 13, 14 and 11 days, for first, second and third courses, respect-
Four patients with metastatic breast cancer did not receive a third course of tCTC and three patients with metastatic ovarian cancer did not receive a second course of tCTC due to excessive toxicity in previous courses.

Table 2 shows an overview of the toxicity encountered in the patient population. In general, few severe toxic events occurred and no toxic deaths were encountered. VOD was seen after a second course of CTC, after a third course of tCTC and after a second course of tCTC. This latter patient also developed grade 4 diarrhoea after this course. Haemorrhagic cystitis was seen after a single course of CTC and twice after a second course of tCTC. In both latter patients, a third course of tCTC was administered without cyclophosphamide without further severe complications. In general, peak levels of serum bilirubin, ALAT, ASAT and creatinine were detected 2 days after chemotherapy (the day of the PBPC transplantation) and normalised within a few days. Grade 4 toxicity of these parameters was only detected in patients who developed VOD. Grade 3 mucositis was only observed after single courses of CTC. For patients who received multiple courses of tCTC, severity of mucositis in general increased after the later courses compared to the first course.

Blood samples were collected from all patients. Some patients, however, withdrew informed consent for the pharmacokinetic part of the study during treatment and, therefore, pharmacokinetic data were available for 77 of the 83 administered courses. From first courses, data were available. Thiopeta, carboplatin and cyclophosphamide data were available for all patients. Tepa and 4-hydroxycyclophosphamide levels were determined in 35 and 38 of the 46 patients, respectively. Phosphoramide mustard levels were available for the last seven patients (15 courses) entered. Due to instability of tepa, 4-hydroxycyclophosphamide and phosphoramide mustard in plasma, it was not possible to determine these metabolites in the population retrospectively.

The results of the population pharmacokinetic analyses of carboplatin, thiopeta and tepa, and cyclophosphamide and metabolites are summarised in Tables 3, 4 and 5, respectively. Based on these population analyses, individual AUC values were calculated for all drugs and metabolites. These AUC values are summarised in Table 6.

Relationships between toxicity of the first course and the pharmacokinetics were tested. Significant relationships were identified between ALAT and the combined thiopeta and tepa AUC and between ASAT and the thiopeta AUC (Table 7). No other significant relationships between toxicity after the first course and pharmacokinetics were identified.

For several classes of toxicity (e.g. mucositis and ototoxicity), the severity increased during subsequent chemotherapy courses. The maximal toxicity grade of all courses was scored for each patient within these classes. Since the number of toxic events was relatively low, the NCI-CTC scores were transformed to a dichotomous scale (e.g. no toxicity versus toxicity of any grade). Relationships between the cumulative AUC of the different compounds and these toxicities were tested with logistic regression. A significant relationship was found between neuropathy (no toxicity versus toxicity of any grade) and the cumulative carboplatin AUC ($P = 0.04$). When,
however, cisplatin pretreatment was taken into account, this relationship was not significant ($P = 0.14$).

Since the AUC of the first course may be a prognostic factor for the development of severe toxicity after subsequent courses, relationships between the AUC of the different compounds of the first course and toxicity were tested. The number of courses administered before maximal toxicity was observed was considered as a possible confounding factor and was therefore included in the logistic regression model. A relationship was found between mucositis (grade $<1$ versus grade $\geq 2$) and the tepa AUC. Relationships between carboplatin AUC and ototoxicity and neuropathy (no toxicity versus toxicity of any grade) were identified ($P = 0.011$ and $P = 0.05$, respectively). When these relationships were corrected for cisplatin pretreatment, only the relationship between carboplatin AUC of the first course and ototoxicity remained significant (Table 7). Since the AUC of the parent compounds and the metabolites may be correlated to the absolute dose administered, the toxicities, as shown in Table 7, were also related to the absolute dose of the compound or its parent. Again, significant relationships were found except for the relationship between mucositis and the tepa AUC, but the $P$ values were in all cases higher than for the corresponding relationships between AUC and toxicity. Figure 2 shows graphical representations of the significant relationships between toxicity and pharmacokinetics.

Data for phosphoramide mustard and 4-hydroxycyclophosphamide were available for one and two patients, respectively, who experienced VOD. Figure 3 shows a comparison of the AUC of these compounds during the first course between patients with and without VOD. Although not statistically significant, an indication of a relationship between the AUC of 4-hydroxycyclophosphamide and phosphoramide mustard and VOD was found. A $P$ value of 0.06 was found for the relationship between the cumulative 4-hydroxycyclophosphamide AUC and occurrence of VOD. The patient with the

### Table 3. Population pharmacokinetic parameters of carboplatin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSD (%)</th>
<th>IIV (%)</th>
<th>IOV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance (l/h)</td>
<td>7.44</td>
<td>5.4</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Volume of distribution (l)</td>
<td>10.4</td>
<td>6.7</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Distribution microconstant $k_{12}$ (l/h)</td>
<td>0.672</td>
<td>13</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Distribution microconstant $k_{21}$ (l/h)</td>
<td>0.944</td>
<td>9.3</td>
<td>19</td>
<td>ND</td>
</tr>
<tr>
<td>Residual proportional error (%)</td>
<td>12.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual additive error (µM)</td>
<td>0.723</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RSD, relative standard deviation of estimate; IIV, interindividual variability; IOV, interoccasion variability; ND, not determined.

### Table 4. Population pharmacokinetic parameters of thiotepa and tepa [27]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>RSD (%)</th>
<th>IIV (%)</th>
<th>IOV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clearance thiotepa (l/h)</td>
<td>34.5</td>
<td>9.8</td>
<td>16</td>
<td>15</td>
</tr>
<tr>
<td>Volume of distribution thiotepa (l)</td>
<td>45.8</td>
<td>7.2</td>
<td>74</td>
<td>19</td>
</tr>
<tr>
<td>Distribution microconstant $k_{12}$ thiotepa (/h)</td>
<td>0.273</td>
<td>20</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Distribution microconstant thiotepa $k_{21}$ (/h)</td>
<td>0.459</td>
<td>41</td>
<td>47</td>
<td>ND</td>
</tr>
<tr>
<td>Fraction converted to tepa/volume of distribution tepa (/l)</td>
<td>0.0298</td>
<td>2.0</td>
<td>36</td>
<td>26</td>
</tr>
<tr>
<td>Elimination rate constant tepa (/h)</td>
<td>0.584</td>
<td>1.8</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>Distribution microconstant tepa $k_{12}$ (/h)</td>
<td>2.79</td>
<td>22</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Distribution microconstant tepa $k_{21}$ (/h)</td>
<td>0.855</td>
<td>8.8</td>
<td>24</td>
<td>ND</td>
</tr>
<tr>
<td>Residual proportional error thiotepa (%)</td>
<td>27.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual additive error thiotepa (µM)</td>
<td>0.0631</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual proportional error tepa (%)</td>
<td>20.9</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RSD, relative standard deviation of estimate; IIV, interindividual variability; IOV, interoccasion variability; ND, not determined.
second highest phosphoramide mustard AUC during the first course, developed severe haemorraghic cystitis after the second course.

**Discussion**

In high-dose chemotherapy with the use of PBPC transplantation, haematological toxicity is not dose limiting. However, due to the high doses employed, other severe and sometimes life-threatening toxicities are encountered and the occurrence of these toxicities was mainly unpredictable thus far. Therefore, the major aim of the current study was to identify the relationships between pharmacokinetics of the different agents and their metabolites and toxicity in a typical high-dose regimen.

In this study, we aimed to establish the pharmacokinetics of all agents and relevant metabolites in the (t)CTC regimen. Data were not available on all planned time points and/or treatment days for all patients. Population pharmacokinetic analyses were used in order to establish the pharmacokinetics of all agents. With these methods it proved possible to obtain individual pharmacokinetic parameters even from patients with limited data available.
Table 7. Overview of significant relationships between toxicity and pharmacokinetics

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Grade</th>
<th>Pharmacokinetic parameter</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALAT in first course</td>
<td>Any grade</td>
<td>Thiotepa plus tepa AUC of first course</td>
<td>0.016\textsuperscript{a}</td>
</tr>
<tr>
<td>ALAT in first course</td>
<td>Grade ( \leq 1 ) versus ( \geq 2 )</td>
<td>Thiotepa plus tepa AUC of first course</td>
<td>0.008\textsuperscript{b}</td>
</tr>
<tr>
<td>ASAT in first course</td>
<td>Any grade</td>
<td>Thiotepa AUC of first course</td>
<td>&lt;0.0001\textsuperscript{a}</td>
</tr>
<tr>
<td>ASAT in first course</td>
<td>Grade ( \leq 1 ) versus ( \geq 2 )</td>
<td>Thiotepa AUC of first course</td>
<td>0.005\textsuperscript{b}</td>
</tr>
<tr>
<td>Mucositis (maximal grade during multiple courses)</td>
<td>Grade ( \leq 1 ) versus ( \geq 2 )</td>
<td>tepa AUC of first course\textsuperscript{c}</td>
<td>0.025\textsuperscript{b}</td>
</tr>
<tr>
<td>Otoxicity (maximal grade during multiple courses)</td>
<td>No toxicity versus grade ( \geq 1 )</td>
<td>Carboplatin AUC of first course\textsuperscript{d}</td>
<td>0.028\textsuperscript{b}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Tested with the Kruskal–Wallis test.
\textsuperscript{b}Tested with logistic regression.
\textsuperscript{c}Total number of courses administered before maximal toxicity was observed included in logistic regression model.
\textsuperscript{d}Total number of courses administered before maximal toxicity was observed and cisplatin pretreatment included in logistic regression model.

Figure 2. Graphical representation of the relations between toxicity and pharmacokinetics. (A) Combined thiotepa and tepa area under the concentration–time curve (AUC) of the first course and elevation of alanine aminotransferase (ALAT) after the first course; (B) Thiotepa AUC of first course and elevation of aspartate aminotransferase (ASAT) after the first course; (C) tepa AUC of first course and maximal grade of mucositis; (D) Carboplatin AUC of first course and maximal grade of ototoxicity.
Although the number of toxic events was relatively low in the population included, several relationships between toxicity and pharmacokinetics were identified. Severe mucositis is one of the major complications of high-dose thiotepa therapy [2]. In previous studies, relationships between thiotepa pharmacokinetics and mucositis have been identified [16]. In the study by Hussein et al. [16], however, tepa pharmacokinetics were not evaluated. In the study of Przepiorka et al. [17], relationships between the combined thiotepa and tepa AUC or tepa peak levels exceeding 1750 ng/ml and any toxicity of grade ≥2 were found. In the current study, a relationship between severe mucositis after multiple courses and tepa AUC of the first course was identified. Tepa has a considerably longer half-life than thiotepa and therefore the AUC of tepa in general exceeds the AUC of thiotepa. These results indicate that tepa may play an important role in the activity and toxicity of thiotepa. At doses over 1000 mg/m², transient elevations of liver enzymes and bilirubin have been encountered [30]. In the current study, relationships between elevations of liver enzymes and the thiotepa and tepa AUC were identified, which may be in agreement with the results of Wolff et al. [30].

Although the relationship between VOD and 4-hydroxycyclophosphamide AUC was not statistically significant, it may be very important clinically since VOD is one of the major causes of toxic death after high-dose chemotherapy [31]. The role of the activated metabolites of cyclophosphamide in the development of VOD has been studied in vitro [32]. In the presence of hepatocytes, cyclophosphamide proved toxic to sinusoidal endothelial cells, while 4-hydroxycyclophosphamide was toxic in the absence of hepatocytes. Toxicity was mediated by a depletion of intracellular glutathione [32]. Since, cyclophosphamide is activated mainly in the liver to 4-hydroxycyclophosphamide, high concentrations of 4-hydroxycyclophosphamide locally may lead to liver damage and ultimately VOD. In our study, the number of patients with VOD was low and therefore it was impossible to demonstrate a significant relationship. Since VOD is less frequently encountered after single agent cyclophosphamide [2, 31], more factors may be related to the development of VOD. In previous studies, elevated transaminase levels prior to the conditioning regimen proved a prognostic factor for the development of VOD [31, 33]. In our study, a relationship between elevated liver function test and thiotepa and tepa AUC was identified, which may suggest a role for thiotepa in the development of severe hepatotoxicity and VOD.

The role of phosphoramide mustard in the development of VOD remains unclear, since phosphoramide mustard data were only available for seven patients. Of these patients, one had a substantially higher AUC of phosphoramide mustard during the first course compared to the other patients, which suggests a role for phosphoramide mustard in the development of VOD.

![Figure 3](image-url)
No relationship was identified between parent cyclophosphamide and toxicity, which may be explained by the fact that cyclophosphamide is a prodrug. In previous studies, however, relationships between cyclophosphamide AUC and cardiotoxicity have been identified [14, 15]. In a large cohort of patients, Nieto et al. [34] failed to demonstrate any relationship between toxicity and the pharmacokinetics of parent cyclophosphamide. These findings, along with our own, suggest that it may be important to determine levels of the activated metabolites 4-hydroxycyclophosphamide and phosphoramid mustard.

The relationship between the rate of bone marrow recovery after PBPC transplantation and pharmacokinetics was not studied. In a previous study, we have shown that these effects are strongly related to the size of graft re-infused (number of granulocyte/macrophage colony forming units and number of CD34 positive cells) [35].

The relationships between severe toxicity and exposure suggest a role for pharmacokinetic guided dosing. Individual dosing may avoid excessively high exposures and may prevent severe toxicity. In contrast, patients with low exposures may benefit from an increased dose in the absence of a higher risk of severe toxicity. For busulfan, a pharmacokinetic guided dosing strategy led to a strong reduction in the incidence of VOD [36]. Cyclophosphamide metabolism shows, however, auto-induction and is inhibited by thiopeta in the CTC regimen. Nieto et al. [34] showed that the AUC of cyclophosphamide of the first day of a course was not predictive for the AUC of a complete course, which complicates pharmacokinetic guided dosing of cyclophosphamide during a course. In the pharmacokinetic model used in this study the effects of auto-induction and the interaction with thiopeta were included, which may enable pharmacokinetic guided dosing.

In conclusion, the complex pharmacokinetics of the different agents and their metabolites have been established and several relationships between the pharmacokinetics and toxicity were identified (between thiopeta and tepa AUC and elevation of transaminases and mucositis and between carboplatin AUC and ototoxicity). These findings may form the basis for further treatment optimisation and dose individualisation in this high-dose chemotherapy combination.

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References

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