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SHORT COMMUNICATION

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Fractionated administration of high-dose cyclophosphamide: influence on dose-dependent changes in pharmacokinetics and metabolism

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Abstract *Purpose*: The alkylating agent cyclophosphamide (CP) is a prodrug that is metabolized to both cytotoxic and inactive compounds. We have previously shown that following dose escalation from conventional-dose (CD) to high-dose (HD) levels; the fraction of the dose cleared by bioactivation is significantly decreased (66% versus 48.5%) in favor of inactivating elimination pathways when the HD is given as a single 1-h infusion. Based on the concept of bioactivating enzyme saturation with increasing doses, we investigated the influence of fractionated application of HD-CP on dose-dependent changes in metabolism. *Patients and methods*: Plasma concentrations of CP (measured by high-performance liquid chromatography, HPLC) and urinary concentrations of CP and its major metabolites (quantified by [^{31}P]-nuclear magnetic resonance spectroscopy; [^{31}P]-NMR spectroscopy), were determined in four patients with high-risk primary breast cancer who received adjuvant chemotherapy including both CD-CP (500 mg/m^2 infused over 1 h) and split HD-CP (50 mg/kg infused over 1 h on each of 2 consecutive days (d): d_1 and d_2. *Results*: (Data are given as mean values for CD and d_1/d_2 of HD, respectively). Systemic clearance (CL) of CP was similar during CD and d_1 of HD, but significantly increased on d_2 of HD (CL: 83 and 78/115 ml/min; $P < 0.01$ for d_1 versus d_2). The latter was translated into an increase in formation CL of both active (+16.4 ml/min) and inactive metabolites (+17.6 ml/min) and reflects autoinduction of metabolism. As compared with CD-CP, no statistically significant decrease was observed in the relative contribution of bioactivation CL to overall CL during both days of HD (63% versus 57%/53%). Recovery of intact CP in 24-h urine corresponded to 24%, 29%, 22% of the dose ($P < 0.05$ for d_1 versus d_2 of HD). *Conclusions*: Following dose escalation of CP, dividing the high dose over 2 days instead of one single infusion may favorably impact the metabolism of CP in terms of bioactivation. In addition, on day 2 of a split regimen, renal elimination of CP is decreased, which implies that more drug is available for metabolism.

Key words Cyclophosphamide · High dose · Pharmacokinetics · Application schedule

Introduction

One of the most commonly used drugs in high-dose chemotherapy is the alkylating anticancer agent cyclophosphamide (CP), a prodrug that requires enzymatic bioactivation to manifest its anticancer cytotoxic activity. The fate of CP following its administration is very complex, as it undergoes a sequence of activating and inactivating pathways (a current understanding of the metabolism of CP has been summarized in [10]). In brief, bioactivation is initiated by hydroxylation of CP to 4-hydroxycyclophosphamide, which is in equilibrium with its tautomer aldophosphamide. Alternatively, a fraction of the parent compound is inactivated to dechloroethylcyclophosphamide by side-chain oxidation or is excreted unchanged in urine. The unstable intermediates 4-hydroxycyclophosphamide/aldophosphamide, in turn, are converted into the active alkylating species phosphoramide mustard, but they may also be detoxified by

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oxidation to carboxyphosphamide and ketocyclophosphamide.

We have recently described the influence of dose escalation on the pharmacokinetics and metabolism of CP [10]. An intraindividual comparison of metabolic profiles during conventional-dose (CD) and high-dose (HD) therapy clearly demonstrated that following dose escalation of CP from standard to transplant levels, the fraction of the dose cleared by bioactivation is significantly decreased (66% versus 48.5%) in favor of inactivating elimination pathways, thereby leading to an unfavorable relationship between the increase in scheduled dose and real dose intensification. Analogous to CD therapy, HD-CP was given as a single infusion over 1 h (which is a clinically used application schedule [4, 30]), and the observed decrease in bioactivation clearance during the HD regimen was assumed to be due to saturable kinetics of the microsomal enzymes responsible for 4-hydroxylation, which is in fact supported by in vitro data [9, 25]. Consequently, fractionated administration of HD-CP could favorably alter the clinical pharmacokinetics of CP in terms of bioactivation. In addition, splitting of the dose could lead to increased exposure to bioactivated metabolites due to autoinduction of metabolism, which is known to occur with repeated doses of CP [3, 16, 17, 22, 29, 31]. We therefore investigated the pharmacokinetics and metabolism of CD-versus HD-CP, with the HD being divided into equal fractions given on 2 consecutive days. Analogous to our previous work comparing CD- with single HD-CP [10], the pharmacokinetics of CP during CD and split-HD therapy were investigated in the same patient. This approach allows reliable assessment of dose-dependent changes by excluding the influence of interpatient variability. In addition, the present investigation was performed with the identical analytical techniques as used in our previous study on single HD-CP [10], thus allowing comparison of the data.

Patients and methods

Patient population

Pharmacokinetic investigations were performed in four women with operable stage II–III primary breast cancer. The patients were the last ones in our hospital (following the 12 patients of our previous study [10]) who had been scheduled for the postoperative adjuvant chemotherapy protocol including both CD- and HD-CP [10]. Their mean age (± SD) was 45 ± 6 (range 40–54) years and their mean body weight was 73 ± 15 (range 63–95) kg. Inclusion criteria were the involvement of more than ten axillary lymph nodes, no metastatic disease, and adequate function of the kidney, liver, heart, and lung [10]. The study protocol was approved a priori by the ethics committee of the Robert Bosch Hospital (Stuttgart, Germany) according to the ethical guidelines of the Declaration of Helsinki (Helsinki, Finland), and written informed consent was obtained from each patient.

Treatment

The adjuvant chemotherapy protocol consisted of four cycles of CD (CDI–IV) and one final course of HD combination therapy with CP, doxorubicin, and etoposide, respectively, as previously reported [10]. However, the HD regimen was modified as follows: instead of being given as one single infusion, the total dose of CP (100 mg/kg) was divided over 2 consecutive days (d; $d_{9–10}$ of the original schedule), with half of the total amount (i.e., 50 mg/kg) being given on each of both days. As a consequence, the continuous infusion of mesna (150 mg/kg body weight) was prolonged from 48 to 72 h ($d_{9–11}$ of the HD regimen) and autologous hematopoietic stem cells were reinfused on d_{13} instead of on d_{12}. In each patient, pharmacokinetic investigations of CP were carried out in cycle III of CD chemotherapy (CP: 500 mg/m^2 as a 1-h infusion) and during both days of HD-CP (CP: 50 mg/kg as a 1-h infusion on each of 2 consecutive days: d_1 and d_2 of HD-CP).

Hydration was provided with a volume of 1.5 and 3 l of saline or Ringer solution/day during CD-CP and during each day of HD-CP, respectively. Concomitant medication included antiemetics (metoclopramide, ondansetron, dexamethasone), sedatives (triflupromazine), and, during HD therapy only, prophylactic antibiotics (fluconazole, acyclovir, colistin, sulfamethoxazole/trimethoprim, amphotericin B suspension) and antacids (omeprazole). The impact of possible drug interactions on pharmacokinetics of CP has been discussed in detail in a recent publication [10].

Blood and urine sampling

On each study day, blood samples (5 ml) were obtained from an indwelling cannula at time points 0, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 20, and 24 h after the beginning of the CP infusion. In addition, urine was collected for up to 24 h after the onset of drug administration. Blood and urine specimens were handled as reported previously [10].

Analytical methods

Plasma levels of CP were measured by high-performance liquid chromatography (HPLC). Urinary concentrations of CP and its three inactive metabolites dechloroethylcyclophosphamide (dechloroethylCP), ketocyclophosphamide (ketoCP), and carboxyphosphamide were quantified by [^{31}P]-nuclear magnetic resonance (NMR) spectroscopy. Both methods have been described in detail earlier [10].

Pharmacokinetic analysis

The area under the curve (AUC; $AUC_{0-\infty}$ during CD-CP and $AUC_{0–24h}$ during d_1 and d_2 of HD-CP, respectively), volume of distribution (V), elimination half-life ($t_{1/2}$), systemic clearance (CL), renal clearance (CL_{ren}), and metabolic clearance (CL_{met}) were calculated as previously described [10]. CL_{met} to the inactive metabolites dechloroethylCP, ketoCP, and carboxyphosphamide was calculated directly as CL_{met} = amount of metabolite excreted/AUC-CP, as these metabolites are ultimately excreted in urine. CL_{met} to the unstable intermediate 4-hydroxycyclophosphamide (4-hydroxy CP) and the fraction that is further converted to phosphoramide mustard were calculated indirectly by metabolic differences. According to the metabolic scheme of CP, CL_{met} 4-hydroxyCP was assumed to correspond to CL_{sys}-CL_{ren}-CL_{met} dechlorethylCP (previous work has shown that the biliary excretion of CP and its metabolites is negligible [3]). The fraction of CL_{met} 4-hydroxyCP that was not detoxified (and thereby recovered in urine as ketoCP and carboxyphosphamide) was assumed to represent CL_{met} to reactive metabolites of the bioactivation pathway and was termed $CL_{met^{\Delta}}$ (bioactivation).

Statistical analysis

All data are presented as mean values ± SD and as median values, respectively. Differences in the pharmacokinetics of CP between

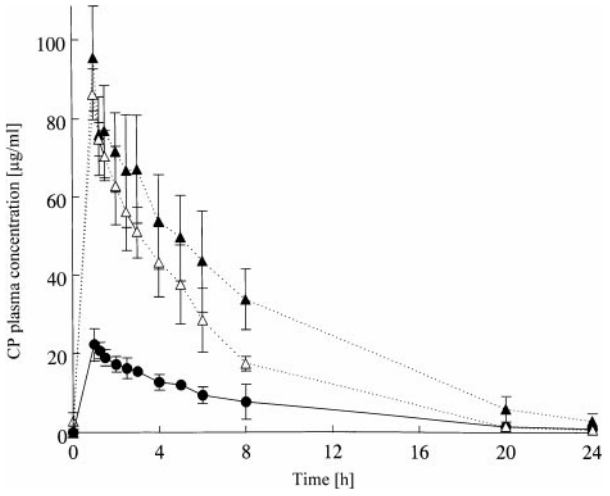

Fig. 1 Mean plasma concentration-time curves generated for CP during CD (●, 500 mg/m^2 given as a 1-h infusion) and HD therapy (50 mg/kg given as a 1-h infusion on each of 2 consecutive days: ▲ day 1, △ day 2): Data represent mean value ± SD ($n = 4$)

CD therapy and both days of HD therapy, respectively, were evaluated by two-sided paired *t*-tests. Correlation between the individual 24-h urinary volume and the amount of CP excreted unchanged in 24-h urine was analyzed by linear regression. *P* values below 0.05 were considered to be significant.

Results

Mean serum-concentration time profiles obtained for CP during CD therapy and the 2 days (d_1, d_2) of HD therapy are shown in Fig. 1. Despite the administration of identical doses of CP on both days of the HD regimen, plasma concentrations achieved on d_2 were reduced by a mean of 26% (range 3–79%) in comparison with the corresponding time points on d_1.

The administered doses and pharmacokinetic parameters of CP during CD therapy and both days of HD therapy are summarized in Table 1. A comparison of CD with d_1 of split-HD therapy (i.e., following an approximately 4-fold dose escalation) showed no difference in the dose-corrected AUC, V, CL, and $t_{1/2}$ of CP. In addition, there was no statistically significant change in the individual clearances of CP. The relative contribution of the primary and the final step of the bioactivation sequence [CL_{met} 4-hydroxyCP and $CL_{met^{\Delta}}$ (bioactivation), respectively] to overall CL during d_1 of HD therapy was only moderately lower than that observed during previous CD therapy (d_1 of HD versus CD: 66% versus 72% and 57% versus 63%, respectively).

As compared with CD and d_1 of HD, considerable modifications in the pharmacokinetics of the parent compound were observed on d_2 of the HD regimen, indicating a faster elimination of CP from plasma (Table 1); there was a significant decrease in the dose-corrected AUC (d_1 versus d_2 of HD: 220 versus 147 [µg h/ml g], $P < 0.05$) and in the elimination half-life (d_1 versus d_2: 4.6 versus 3.2 h, $P < 0.01$) in parallel with a significant increase in systemic CL (d_1 versus d_2: 78 versus 115 ml/min, $P < 0.01$). The latter was nearly exclusively due to an increase in nonrenal CL (d_1 versus d_2: 55.8 versus 89.7 ml/min, $P < 0.01$), whereas renal CL increased only moderately (d_1 versus d_2: 22.2 versus 25.3 ml/min). Induction of metabolism resulted in a significant rise in CL_{met} of all primary (CL_{met} 4-hydroxyCP: +31 ml/min, CL_{met} dechloroethylCP: +4 ml/min) and secondary [$CL_{met^{\Delta}}$ (bioactivation): +16.4 ml/min, CL_{met} ketoCP: +1 ml/min, CL_{met} carboxyphosphamide: +12.6 ml/min) metabolic pathways and was translated almost equally into an increase in formation CL of both bioactivated and inactive metabolites (+16.4 and +17.6 ml/min, respectively)]. As compared with d_1 of HD-CP and CD-CP, respectively, the relative contribution of 4-hydroxyCP formation to overall CL was not significantly altered (70% versus 66% and 72%, respectively). In addition, only a minor decrease was

Table 1 Pharmacokinetic parameters of CP in 4 patients with breast cancer during CD and HD chemotherapy. Numbers in parentheses represent the fraction of the dose cleared by the individual pathway, calculated as the ratio: CL pathway/CL CP; mean ± 1 SD

	CD 500 mg/m^2 over 1 h	HD (day 1) 50 mg/kg over 1 h	HD (day 2) 50 mg/kg over 1 h
Dose of CP (g)	0.9 ± 0.05	3.2 ± 0.3	3.2 ± 0.3
C_{max} (µg/ml)	22.7 ± 4.0	96 ± 17	84 ± 7
AUC/g CP [µg h/(ml g)]	204 ± 31*	220 ± 40*	147 ± 18
V(l/kg)	0.48 ± 0.09	0.41 ± 0.07	0.44 ± 0.05
$t_{1/2}$ (h)	5.1 ± 0.5**	4.6 ± 0.7**	3.2 ± 0.4
CL[a] (ml/min)	83 ± 12*	78 ± 14**	115 ± 14
1. CL_{ren} (ml/min)	19.9 ± 2.6 (24 ± 4)	22.2 ± 1.3 (29 ± 5)	25.3 ± 1.3 (22.3 ± 4)
2. CL_{met} dechloroethyl CP (ml/min)	3.1 ± 1.3**(4 ± 1.1)	4.2 ± 0.8** (5.5 ± 1.6)	8.2 ± 0.9 (7.3 ± 1.7)
3. CL_{met} 4-hydroxyCP[b] (ml/min)	60.0 ± 10.5 (72 ± 3)	51.5 ± 15.1** (65.5 ± 7)	81.5 ± 17.2 (70.3 ± 5)
3A. CL_{met} ketoCP (ml/min)	1.3 ± 0.5* (1.5 ± 0.6)	1.3 ± 0.3** (1.6 ± 0.2)	2.3 ± 0.4 (2.0 ± 0.2)
3B. CL_{met} carboxyphosphamide (ml/min)	6.9 ± 4.2* (7.9 ± 4)	5.1 ± 4.9* (6.6 ± 6)	17.7 ± 6.6 (15.4 ± 5)
3C. $CL_{met^{\Delta}}$ (bioactivation) (ml/min)	51.8 ± 6.4 (63 ± 2)	45.1 ± 16.0* (57 ± 10)	61.5 ± 15.4 (53 ± 7)
CP in 24-h urine (% of dose)	24.3 ± 3.7	29.1 ± 5.4*	22.4 ± 3.8
Urinary volume (l/24 h)	4.0 ± 0.7	4.1 ± 0.6	5.5 ± 1.9

*$P < 0.05$; *$P < 0.01$ for CD- and/or day 1 of HD- versus day 2 of HD-CP, respectively
[a]Sum of CL (1.0–3.0); [b]Sum of CL (3A–3C), as described in Patients and methods

observed for the fraction of the dose that was finally converted to active metabolites (53% versus 57% and 63%, respectively), which was due to a relative increase in formation of carboxyphosphamide.

Following the application of the first half of HD-CP, the percentage of the dose that was excreted unchanged in urine was moderately higher than that observed during previous CD therapy (29.1% versus 24.3%, nonsignificant difference; Table 1). In contrast, on d_2 of the HD regimen, urinary recovery of the parent compound decreased, being significantly lower than on d_1 (29% versus 22%; $P < 0.05$) and in the same range as during CD therapy.

Discussion

The basic concept of HD therapy is increased exposure to therapeutic agents. Ensuring a maximization of the therapeutic index is a special challenge in the design of HD regimens that include anticancer prodrugs like CP, since pharmacokinetic factors such as the capacity of the bioactivating enzymes may modulate the dose intensity. This problem was highlighted in our previous study, which demonstrated that the application of HD-CP as a single short-term infusion resulted in a significant decrease in the bioactivation clearance when compared with CD therapy [10]. As a consequence, we investigated whether modulation of the application schedule of HD-CP in the sense of fractionated administration could favorably alter the pharmacokinetics of CP in terms of bioactivation.

The present data suggest that following an 8-fold dose escalation of the CP, the fraction of the dose cleared by bioactivation is not significantly altered when the HD is divided over 2 days. In addition, split-HD-CP differs from single-HD-CP in that half of the dose is converted into active metabolites significantly faster and the excretion of unchanged CP via the kidneys is reduced.

The pharmacokinetic parameters recorded for CD-CP in the 4 patients of the present study are in complete agreement with the corresponding data obtained during identical treatment in the peviously investigated 12 patients with breast cancer [10]. In these women, pharmacokinetic parameters of CP remained unchanged following an 8-fold dose escalation [10]. Accordingly, in the present study, no difference in the pharmacokinetics of the parent compound was observed following an intermediate (i.e., 4-fold) increase of dose on d_1 of HD-CP. However, the clearance profile of CP was differently affected, depending on the magnitude of dose escalation: following a single infusion of HD-CP the fraction of the parent compound cleared by 4-hydroxylation was significantly reduced in favor of renal CL and formation of dechloroethylCP, when intraindividually compared with CD-CP (77% versus 65% of CL, $P < 0.01$). This decrease in the first step of the bioactivation sequence contributed to a reduction in final bioactivation CL from 66% to 48.5% of CL [10]. In contrast, with half of the total HD on d_1 of the split regimen, the relative contribution of 4-hydroxylation was only moderately lower than that seen during previous CD therapy (66% versus 72%) and, consequently, bioactivation CL was not affected in a significant way (57% versus 63%). On the basis of in vitro data, which indicate saturable kinetics for 4-hydroxylation at high concentrations [8, 9, 25], the data suggest that a lowering of peak concentrations by splitting of the HD may favorably impact the bioactivation of CP.

As compared with CD, renal elimination of intact CP was only moderately increased on d_1 of HD therapy (24% versus 29% of the dose). This contrasts with the significant rise in urinary recovery of the parent compound observed in our previous study comparing CD-CP with single-HD-CP (19% versus 30% [10]). However, this change was not attributed to the different dose levels but rather to the intensification of hydration during the HD regimen. Our observation that the renal elimination of CP does not depend on the dose but rather on the individual urine flow (which influences the extent of tubular drug reabsorption) is in accordance with the results reported by Bagley et al. [3], who found no correlation between the urinary excretion of CP and a given drug amount. Additional evidence in support of this assumption is provided by our finding of a highly significant correlation between the individual 24-h urinary volume and the percentage of the administered CP dose that was recovered unchanged in 24-h urine. The latter was independently of the dose, which varied from 500 mg/m^2 (CD therapy) to 50 mg/kg (d_1 of the split-HD regimen) and 100 mg/kg (single infusion of HD-CP). Figure 2 summarizes the data recorded for 16 patients during our prior investigation ($n = 12$) [10] and during the present study ($n = 4$; $r = 0.69$, $P < 0.001$). Due to the intensification of hydration during HD

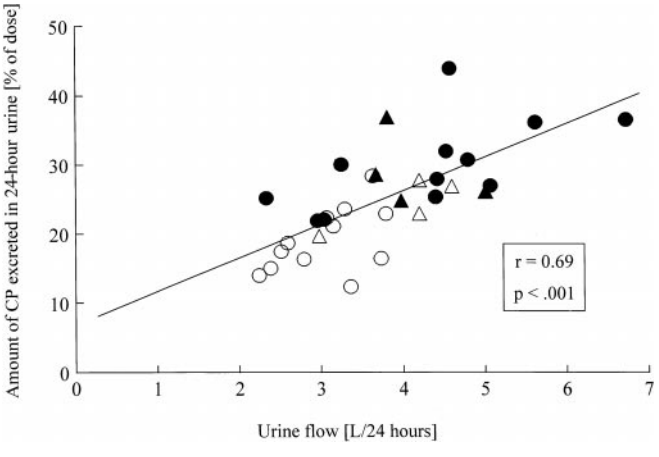

Fig. 2 Correlation between individual urinary flow (l/24 h) and the amount of CP excreted unchanged in 24-h urine (% of dose). Dose of CP: + (1) *white symbols* = 500 mg/m^2 given as a 1-h infusion: △ ($n = 4$, present study), ○ ($n = 12$ [10]); (2) ▲ 50 mg/kg given as a 1-h infusion ($n = 4$, present study); (3) ● 100 mg/kg given as a 1-h infusion ($n = 12$ [10])

therapy, the urine flow is increased, which in turn results in increasing amounts of the dose being eliminated unchanged via the kidneys thereby giving an example of supportive treatment influencing the pharmacokinetics of CP in an unfavorable way).

Interestingly, following administration of the second half of HD-CP on d_2, the percentage of the drug eliminated unchanged in urine was significantly lower than that seen on d_1, although the mean 24-h urinary flow was even increased (Table 1). A similar observation (i.e., a decrease in the urinary recovery of intact CP following administration of the second dose) has been reported by other authors [26, 28]. This minor but favorable change (the amount of CP eliminated via the kidneys is definitively not available for metabolic activation) most likely results from the enhanced metabolic activity observed on d_2 of the split-HD schedule. Autoinduction of metabolism, causing an increase in nonrenal CL and a decrease in $t_{1/2}$ but no change in apparent V is a well-known phenomenon occurring with two or more repeated doses of the oxazaphosphorines CP and ifosfamide [3, 6, 15–7, 19, 20, 22, 23, 26, 29, 31]. Our data concerning the pharamcokinetic changes of CP occurring with administration of the second dose (i.e., an increase in systemic CL of 47% and a decrease in $t_{1/2}$ of 30%) are in the range of those reported in the literature [17, 26, 29]. Previous clinical studies investigating the effect of autoinduction on the metabolism of CP, however, have focused on the pharmacokinetics of the parent compound [3, 15–17] and, in some cases, on the formation of 4-hydroxyCP [26, 29, 31] and/or phosphoramide mustard [22, 29], whereas the influence of autoinduction on the overall metabolism of CP has not been published so far.

In contrast to several animal studies, which show even suppression of microsomal enzymes by CP [11, 18, 21], recent in vitro data actually demonstrate the induction of cytochrome P450 enzymes by CP and ifosfamide in human hepatocytes [14], thereby providing a molecular basis for the observed clinical effects. Accordingly, the two initial metabolic steps of CP (i.e., 4-hydroxylation and dechloroethylation), both of which are catalyzed by cytochrome P450 enzymes [7, 12, 13], were significantly enhanced (1.6- and 1.96-fold, respectively). The increase in metabolic CL of the three non-cytochrome P450-dependent pathways subsequent to 4-hydroxylation (i.e., the formation of ketoCP, carboxyphosphamide, and phosphoramide mustard [27]) may be a direct consequence of the enhanced formation CL of the precursor metabolite. Whether the autoinduction of metabolism results in increased qunatitites of cytotoxic metabolites remains controversial. In accordance with an increased rate of formation, higher peak concentrations of 4-hydroxyCP [29] and the delivered alkylating agent phosphoramide mustard [22] have been reported with administration of the second dose of CP (which might affect drug effects, depending on whether C_{max} is a relevant parameter). However, the AUC of 4-hydroxyCP was found to be either unchanged [29] or increased [26]. In our study the relative contribution of 4-hydroxylation to the overall CL of CP was not altered by autoinduction (d_1 versus d_2: 66% versus 70%). However, renal elimination of the parent compound was significantly reduced on d_2, which implies that more drug is available for metabolism. As compared with CD-CP, also during administration of the second half of HD-CP the relative contribution of 4-hydroxylation was not decreased (70% versus 72% of CL), which contrasts favorably with our data on single-HD-CP [10].

In summary, our comparison of the pharmacokinetic data on HD-CP given either as a single infusion or divided over 2 days suggests that the fraction of the dose that is bioactivated is likely to be higher during the split-application schedule. From the clinical point of view the fractionated administration of HD-CP was tolerated better than a single infusion of the total dose; despite similar comedication, nausea was less frequent and, especially, no CNS side effects (i.e., dizziness, hallucinations) were reported by the parents investigated in this study, whereas the latter symptoms were observed in almost all patients during single-HD-CP [10].

In clinical practice, fractionation of HD-CP over 2 days is a common schedule prior to bone marrow transplantation [16, 22]. However, it is only one among several other application schedules of CP used in the HD setting, e.g., single infusion [4, 27], fractionation of the dose over more than 2 days [5, 24], or continuous infusion over 4 days [1, 2]. Based on the pharmacokinetic data, which suggest that the same total dose of CP given in different application schedules might cause a different exposure to cytotoxic metabolites, comparison of the different application schedules of HD-CP in terms of clinical effects would be of interest and might offer the chance to optimize the therapy with an existing agent by improving the methods of administration.

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