Two issues, the importance of basic research and the challenges of applied clinical research have been emphasized. Due to the carefully chosen topics and the fact that all speakers were allowed enough time for an in-depth discussion, a well-founded debate was possible. We are pleased to present this special issue as an example for interdisciplinary co-operation on a high scientific level.

H. Grunicke
New insights into the clinical pharmacokinetics of trofosfamide

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Abstract. Objective: This study focuses on the pharmacokinetics of trofosfamide (TRO) and metabolites after oral administration of TRO. Methods: Twelve patients with solid tumors and non-Hodgkin lymphomas were treated with 450 mg TRO orally for 7 days. TRO and the stable metabolites ifosfamide (IFO), cyclophosphamide (CYC), 2- and 3-dechloroethylifosfamide (2-DCE, 3-DCE) were determined by GC and the sum of the 4-OH-metabolites was measured by HPLC. Results: A fast metabolism of TRO with a half-life of about 1 h was observed. IFO was the main stable metabolite, whereas CYC was only detected in minor quantities. The peak levels and the AUC of the 4-OH-metabolites were 9.5 and 4.3 times higher than observed after an equimolar IFO dose. Only 6% of the administered dose was recovered in urine within 24 hours as stable metabolites. TRO was under limit of detection. Conclusions: Our results confirm that dechloroethylation of TRO to IFO is a major metabolic pathway. Additionally, we found considerable 4-hydroxylation not shown previously. With respect to the low levels of IFO and CYC observed, the sum of 4-OH-metabolites cannot be explained by hydroxylation of these metabolites only. Hence, we assume a direct 4-hydroxylation of TRO could not be calculated directly, because TRO is only available as an oral formulation. The bioavailability of oral IFO, however, is reported to be almost 100%. Therefore, after normalization of the dose, a bioavailability of 32% for IFO after oral TRO could be calculated. Thus, in contrast to previous reports, direct 4-hydroxylation of TRO seems to be the main metabolic pathway.

Introduction

The oxazaphosphorine derivative trofosfamide (TRO) is an alkylating agent and congener of the well-known anticancer drugs cyclophosphamide (CYC) and ifosfamide (IFO). TRO is more lipophilic than the other oxazaphosphorines and is only available as an oral formulation. The oxazaphosphorines are prodrugs which require hydroxylation at cyclic carbon 4-position by hepatic cytochrome P450 isoenzymes [Wagner et al. 1997]. These unstable 4-OH-metabolites react spontaneously to active phosphoramide-mustard derivatives and acrolein (Figure 1) or they are deactivated by aldehyde dehydrogenases [Kaijser et al. 1994].

According to Hempel et al. [1997], TRO is transformed mainly to IFO in the liver, being dechloroethylated at the exocyclic nitrogen. Only a minor quantity of TRO is converted to CYC. Previous pharmacokinetic studies on IFO have shown that up to 50% are metabolized by side-chain oxidation, which results in the formation of inactive 2-dechloroethylifosfamide (2-DCE) and 3-dechloroethylifosfamide (3-DCE) and the release of cytotoxic chloroacetaldehyde [Börner et al. 2000, Brüggemann et al. 1997, Wagner 1994]. To date, our knowledge about the metabolism of TRO is mainly based on the pharmacokinetic study of Hempel et al. [1997]. Earlier investigations of TRO metabolism used only indirect methods as the 4-nitrobenzylpyridine (NBP) method [Brock 1973], which gives only an estimate of the whole alkylating activity, because this test cannot discriminate between the different metabolites.

The present study was designed to elucidate the metabolic pattern of TRO, because this drug is increasingly used for palliative treatment in various tumors [Blomqvist et al. 1995, Salminen et al. 1997, Wiedemann et al. 1999, Wolff et al. 2000]. Our findings in part confirm the results of Hempel et al. [1997] but they also imply that there is an additional pathway to 4-OH-TRO.
Materials and methods

Patients and study design

Twelve patients (4 male and 8 female patients aged 36 – 68: median 53.8 years) with solid tumors and non-Hodgkin lymphomas were included in the study after having given their written consent. The study was approved by the local Ethics Committee. The patients did not have severe hepatic or renal dysfunction as measured by standard biochemical parameters prior to the commencement of therapy (creatinine < 135 µmol/l, bilirubin < 20 µmol/l, cholinesterase 3,000 – 8,000 U/l, thrombocyte count > 100,000/µl, leukocyte count > 3,000/µl). The performance status of all patients was grade 0 – 2 WHO. None of the patients had received cytostatics within 4 weeks prior to inclusion in the study. The patients did not have severe hepatic or renal dysfunction as measured by standard biochemical parameters prior to the commencement of therapy (creatinine < 135 µmol/l, bilirubin < 20 µmol/l, cholinesterase 3,000 – 8,000 U/l, thrombocyte count > 100,000/µl, leukocyte count > 3,000/µl). The performance status of all patients was grade 0 – 2 WHO. None of the patients had received cytostatics within 4 weeks prior to inclusion in the study. All patients were treated with palliative intention. This study was restricted to 1 treatment cycle, patients with a benefit from the study treatment could be treated with commercially available TRO after the end of the study.

TRO was given over 7 days in a fixed dose of 450 mg/day (50 mg tablets, Asta Medica Oncology, Germany). On the first day of the treatment cycle, the drug was administered as a single dose 30 minutes prior to breakfast. Blood samples were drawn at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8 and 24 h after intake of the tablets. For the assay of IFO, CYC and TRO, blood samples (4.5 ml) were drawn into heparinized syringes (10 U/ml). The samples were centrifuged and the plasma was stored at −20 °C until analysis. Urine samples were collected before TRO administration and over a period of 24 h after oral administration. Additional blood samples of 1 ml for the determination of the 4-OH-metabolites were immediately processed as described below.

GC analysis for TRO and stable metabolites

This method is a modification of the determination of IFO and its stable metabolites published by us in 1993 [Kurowski and Wagner 1993]. After thawing the frozen samples, 400 µl of patient plasma were spiked with 10 µl of the internal standard solution containing dechloroethyl-cyclophosphamide yielding a test concentration of 10 µmol/l. 6 ml dichloromethane were added and the samples were shaken vigorously for 3 min. The organic layer was transferred in a test tube and the extraction procedure was repeated by adding another 2 ml dichloromethane to the plasma samples. The organic layer was evaporated, the residue redissolved in 200 µl ethylacetate and 1 µl was subsequently used for analysis. Immediately after thawing, the urine samples were diluted with aqua dest. (1 : 10) and were treated like the blood samples.

Simultaneous determination of TRO, IFO, CYC and the dechloroethyl-metabolites was performed by means of N/P flame-ionization gas-chromatography (GC/NPFID) using an HP5 column (5% PhMeSilicone) with helium as carrier gas. The temperatures of the oven, injector and detector were set at 150 °C (rate 30 °C/min, 210 °C for 6 min), 205 °C and 300 °C, respectively. The retention times for dechloroethyl-cyclophosphamide, 2-DCE, 3-DCE, IFO, CYC and TRO were 1.6, 2.1, 2.4, 3.6, 3.9, and 6.3 min, respectively. The lower limit of detection at a peak-height to baseline-noise ratio of 3 : 1 for TRO, CYC and IFO was 0.3 µmol/l, for 2-DCE and 3-DCE 0.5 µmol/l.
Assay for 4-OH-metabolites

The assay is based on the release of acrolein reacting with 3-aminophenol to form 7-OH-quinoline, which can be detected fluorometrically [Alarcon 1968]. The results present the sum of the 4-OH-metabolites, because acrolein is liberated from all oxazaphosphorines. A differentiation is not possible between 4-OH-TRO, 4-OH-IFO and 4-OH-CYC. Blood samples were drawn in a tube that contained 1.0 ml of 10% trichloroacetic acid. The samples were shaken vigorously and centrifuged at 3,180 × g for 3 minutes; 0.6 ml reagent solution, consisting of 250 mg 3-aminophenol and 300 mg hydroxylamine hydrochloride in 100 ml hydrochloric acid, was added to a 0.5 ml aliquot of the supernatant, the aliquot was heated at 95 °C for 20 min. The samples were quantified by HPLC with fluorescence detection (excitation 360 nm, emission 505 nm). All patient samples and standards were run in duplicate. The lower limit of detection at 3 : 1 peak-height to baseline-noise ratio was 0.15 μmol/l. Details of the standard preparation and the HPLC assay of fluorescent 7-OH-quinoline as well as representative chromatograms have been published recently [Bohnenstengel et al. 1997, Kaijser et al. 1997].

Pharmacokinetics and calculation

Body surface areas were calculated using the Dubois method. The pharmacokinetic parameters area under the concentration-time curve (AUC), mean residence time (MRT) and terminal elimination half-life (t1/2), were calculated using TopFit V 2.0 [Heinzel et al. 1993] by non-compartmental analysis. Peak concentration (Cmax) and peak time (tmax) values were directly obtained from the concentration versus time profiles.

Results

Pharmacokinetic parameters of TRO, IFO, CYC and the 4-OH-metabolites after oral administration of 450 mg of TRO are summarized in Table 1. Large interindividual variations were observed, especially in the Cmax and AUC values. For TRO, a terminal half-life of about 1 hour was observed whereas the respective values for IFO and CYC were in the expected range of 4 – 8 hours [Boddy and Yule 2000, Wagner 1994]. IFO

Table 1. Pharmacokinetics of trofosfamide and metabolites after oral trofosfamide (450 mg, n = 12), (SD).

<table>
<thead>
<tr>
<th>Drug/metabolite</th>
<th>tmax (h)</th>
<th>Cmax (μmol/l)</th>
<th>AUC0-24h (μmol*h×l⁻¹)</th>
<th>AUC0-∞ (μmol*h×l⁻¹)</th>
<th>MRT (h)</th>
<th>t1/2 (h)</th>
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<tr>
<td>Trofosfamide</td>
<td>1.71</td>
<td>5.53</td>
<td>10.19</td>
<td>10.71</td>
<td>1.85</td>
<td>1.05</td>
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<td></td>
<td>(1.62)</td>
<td>(5.18)</td>
<td>(8.11)</td>
<td>(8.20)</td>
<td>(1.03)</td>
<td>(0.47)</td>
</tr>
<tr>
<td>Ifosfamide</td>
<td>2.17</td>
<td>5.33</td>
<td>41.63</td>
<td>64.31</td>
<td>5.75</td>
<td>7.76</td>
</tr>
<tr>
<td></td>
<td>(1.84)</td>
<td>(2.10)</td>
<td>(32.13)</td>
<td>(39.20)</td>
<td>(2.21)</td>
<td>(4.03)</td>
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<td>Cyclophosphamide</td>
<td>1.92</td>
<td>1.65</td>
<td>8.00</td>
<td>12.99</td>
<td>4.45</td>
<td>5.96</td>
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<td></td>
<td>(1.77)</td>
<td>(1.25)</td>
<td>(7.04)</td>
<td>(11.09)</td>
<td>(2.30)</td>
<td>(4.33)</td>
</tr>
<tr>
<td>4-OH-metabolites*</td>
<td>0.67</td>
<td>1.72</td>
<td>4.32</td>
<td>5.92</td>
<td>2.18</td>
<td>2.49</td>
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<td>(1.19)</td>
<td>(3.50)</td>
<td>(4.75)</td>
<td>(1.21)</td>
<td>(1.57)</td>
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</tbody>
</table>

* = n = 6.
was the predominant metabolite in the plasma of all patients. Its AUC was 5 times higher than the AUC of CYC.

Figure 2 shows the concentration vs. time curves of TRO and the 4-OH-metabolites. The concentration vs. time curve of the 4-OH-metabolites did virtually parallel the parent compound TRO. After 24 hours, the values for TRO were under the limit of detection, while low concentrations of IFO were still detectable. The concentration vs. time curves for IFO and CYC are similar (Figure 3). However, the concentration of CYC was much lower.

Plasma concentrations for the inactive metabolites 2- and 3-DCE mostly ranged between the limit of detection (0.5 µmol/l) and limit of quantification (1.5 µmol/l). Hence, pharmacokinetic calculation was not possible.

The cumulative urinary excretion of IFO, CYC, 2-DCE and 3-DCE measured in a 24-hour period was low (Table 2). Again, IFO was the main metabolite. TRO concentrations were almost negligible at the limit of detection.

### Discussion

In the only detailed pharmacokinetic study on TRO, published so far, Hempel et al. [1997] found that IFO is the predominant metabolite of TRO with a fraction of more than 80% of the metabolites measured. Thus, in consequence, TRO was also denoted as an oral IFO. Our study confirms in part these results with regard to TRO and its metabolism by dechloroethylation mainly to IFO and in a minor quantity to CYC. Dechloroethylation as a first step in TRO metabolism (Figure 1) also results in the release of chloroacetaldehyde which exhibits in contrast to previous opinion [Lewis and Meanwell 1990, Skinner et al. 1993] not only side effects but its own cytotoxic effect on tumor cells in vitro and in vivo [Börner et al. 2000, Brüggemann et al. 1997].

Urinary excretion of TRO and its stable metabolites IFO, CYC, 2-DCE and 3-DCE plays a minor role in the total clearance of TRO because only 6% of the administered dose was recovered as these metabolites within 24 h. These findings again are in accordance with Hempel et al. [1997], who found a cumulative urinary excretion of about 10%. The amount of unchanged TRO was under the limit of detection in urine. Assuming for TRO, a negligible excretion in the feces, as was demonstrated for CYC [Bagley et al. 1973], our results may indicate that the clearance of TRO is nearly a complete metabolic clearance.

The bioavailability of TRO cannot be calculated, because an i.v. formulation is not available. However, the bioavailability of oral IFO previously was found to be almost 100% [Cerny et al. 1986, Wagner and Drings 1986]. Therefore, an estimation of the bioavailability of the main metabolite IFO after oral TRO was possible (Table 3). A linear correlation between dose and AUC and no dose-dependent saturation of enzymatic activation was demonstrated for IFO up to 14

<table>
<thead>
<tr>
<th>Drug/metabolite</th>
<th>µmol</th>
<th>Percent of applied dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trofosfamide</td>
<td>bld*</td>
<td>–</td>
</tr>
<tr>
<td>Ifosfamide</td>
<td>38.45</td>
<td>2.77</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>5.94</td>
<td>0.42</td>
</tr>
<tr>
<td>2-Dechloroethylifosfamide</td>
<td>20.63</td>
<td>1.48</td>
</tr>
<tr>
<td>3-Dechloroethylifosfamide</td>
<td>13.59</td>
<td>0.97</td>
</tr>
<tr>
<td>Total</td>
<td>76.66</td>
<td>5.64</td>
</tr>
</tbody>
</table>

* = below limit of detection.
Thus, it is allowed to normalize the dose of TRO given in this study and of IFO used in a previous study [Wagner and Drings 1986], and to compare the measured AUCs of IFO for calculation of the bioavailability. By this method, we found a bioavailability of only 32% for the metabolite IFO after TRO administration. Using the pharmacokinetic data published by Hempel et al. [1997], a quite similar bioavailability of 34% for IFO could be calculated by us (Table 3). Thus, only one third of a given TRO dose is converted to IFO. These results raise the question whether the absorption rate and bioavailability of TRO is much lower compared to the oxazaphosphorines IFO [Wagner and Drings 1986] and CYC [Wagner and Fenneberg 1984] or there might be a further, so far unattended metabolic pathway. A direct hydroxylation of TRO to 4-OH-TRO (Figure 1) seemed to be a possible explanation.

In the first 6 patients of this study, the 4-OH-metabolites were not measured because the plasma levels of the primary metabolites IFO and CYC were in such a low range that the concentrations of their 4-OH-metabolites would have been below the limit of detection. This was concluded from results of several earlier pharmacokinetic studies with higher i.v.-dosed IFO [Kurowski and Wagner 1993] and CYC [Schuler et al. 1987] resulting in plasma levels of the parent drugs up to 100 times higher than observed after oral TRO in this study. To test our hypothesis of a quantitative important direct hydroxylation pathway of TRO, we subsequently measured the 4-OH-metabolites in a second part of our study. In fact, this was confirmed by finding unexpectedly high levels of 4-OH-metabolites. Though the assay used cannot differentiate between 4-OH-TRO, 4-OH-IFO and 4-OH-CYC, a high percentage of the detected metabolites must be 4-OH-TRO considering the above cited studies [Kurowski and Wagner 1993, Schuler et al. 1987]. In vitro data of Boos et al. [1993] can be interpreted in the same way.

The C<sub>max</sub> levels of the 4-OH-metabolites are 9.5 times and the calculated AUC 4.3 times higher than observed for 4-OH-IFO compared on the basis of an equimolar IFO dose in a previous study [Kurowski and Wagner 1993] (Table 4). Higher peak levels and AUC of the 4-OH-metabolites could be explained in part by the fast metabolism of the parent compound TRO with a terminal half-
life of only 1 h, whereas the metabolic clearance of CYC and IFO is much lower and their terminal half-lives are in the known range of 4–8 h. A further explanation for our results possibly might be a higher stability of 4-OH-TRO than 4-OH-IFO and 4-OH-CYC in blood, which has to be investigated.

In conclusion, our study confirms in part the results from Hempel et al. [1997] with regard to TRO and its metabolism to the stable metabolites IFO and CYC, but adds to the current knowledge that there might be an important direct pathway to 4-OH-TRO.

Acknowledgments

We thank H. Bahrs and M. Vollmert for their skillful technical assistance.

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Effect and interaction of 7-hydroxy methotrexate and methotrexate in AML and ALL patient samples: measured by the thymidylate synthase inhibition assay

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Introduction

Methotrexate (MTX) is one of the most effective anti-tumor drugs in childhood leukemia [Bertino 1993]. In high-dose therapy, its main metabolite 7-OH methotrexate (7-OH MTX) exceeds the concentration of MTX soon after the end of infusion [Erttmann et al. 1985, Milano et al. 1983]. The pharmacodynamic effect of 7-OH MTX itself and its interaction with MTX is not clearly described yet. The thymidylate synthase inhibition assay (TSIA) provides an established method to measure such pharmacodynamic effects ex vivo. Patient samples are incubated with antifolates and the inhibition of the folate depending enzyme thymidylate synthase (TS) is measured.

Materials and methods

We investigated the effect of 7-OH MTX in 7 ALL and 11 AML patient samples by measuring the inhibition of the thymidylate synthase (TS) [Rots et al. 1999]. 7-OH MTX, MTX and combinations of 1 : 1 (1–1) and 7-OH MTX : MTX = 5 : 1 (5–1) (short exposure), respectively 7-OH MTX : MTX = 16 : 1 (16–1) (long exposure) were used in a 3-hour exposure assay (short exposure) with a subsequent drug-free period of 18 hours and in a 21-hour exposure (long exposure). A thymidylate synthase inhibition value of 50% (TSI50) was calculated if a dose response curve was obtained. Assuming linearity of the dose-response curve between the 2 flanking measured activities, the intersection with the inhibition of 50% TS was calculated and the corresponding antifolate concentration declared as TSI(50). Eighteen out of 27 patient samples could be analyzed. All quantifications were done in duplicate.

Additional cytospins were made and were analyzed to confirm the percentage of leukemic blasts, and cell counts were done for the validation of the assays.

Results

Eighteen experiments were analyzed for the 7-OH MTX short exposure in the concentration range from 0.01 μM to 1 mM. No dose-response curve could be obtained in any of these experiments. Median for TS activity was 100% except the concentration of 1 mM (80%) and the concentration of 40 μM (90%). Furthermore, 7 out of 14 experiments showed a minimum of TS activity at the maximum concentration of 1 mM. No difference in TS activity between the different concentrations – analyzed by Friedman test – were observed (p = 0.429; n = 14).

In the 7-OH MTX long-exposure assay concentrations from 0.001 μM to 16 μM were used. None of these 14 patient samples showed a dose-response curve. Medians of TS activity were 100% at all concentrations except 0.001 μM (90%) and 1 μM (90%). All 25% percentiles were below 100%, all 75% percentiles were above 100%. Analysis by non-parametric Friedman test showed no differences in TS activity inhibition between the different concentrations (p = 0.166) showing no effect of 7-OH MTX on the TS activity in the used concentrations neither in short-exposure nor in long-exposure experiments.

The short-exposure assay showed dose-response curves for MTX, 1–1 and 5–1 in the used concentrations from 0.0005 μM to
interaction of 7-OH MTX

500 µM. All 1–1 TSI50 values were lower than the corresponding MTX values and all 5–1 values except one. Median for 1–1 was 0.088 µM (n = 14) and approximately 10-fold lower than for MTX (0.939 µM; n = 14) and 5–1 (0.699 µM; n = 11). Non-parametric analysis by Friedman (n = 11) and subsequent multiple comparison by Dunn’s method showed significant difference between the TSI50 1–1 and MTX (p < 0.05), respectively 1–1, and 5–1 (p < 0.01).

In the long-exposure experiments dose-response curves were seen for MTX, 1–1 and 16–1. Concentrations were used from 16 µM to 0.0002 µM and the TSI50 values were calculated as described above. Median for 1–1 (0.004 µM; n = 13) was about 7 times lower than MTX (0.028 µM; n = 13) and 16–1 (0.027 µM; n = 11). Treatments were statistically significantly different (Friedman, n = 11) and multiple comparison (Dunn) showed differences between MTX and 1–1 (p < 0.05), respectively 16–1, and 1–1 (p < 0.01); 16–1 and MTX did not show any difference in inhibiting the TS (p > 0.05).

Conclusion

These experiments serve for a better understanding of the effect and interaction between 7-OH MTX and MTX and their routinely measured plasma level. The results suggest that 7-OH MTX itself does not influence high-dose therapy on cellular level, but they show an increase in TS activity in equimolar concentrations with MTX. This synergism is reduced by a surplus of the metabolite (5-fold in long exposure, 16-fold in short exposure) but the combination remains as effective as MTX itself.

With the offered caution, in standard chemotherapy (5 g/m² over 24 h) the effect of MTX is at least not degraded by its metabolite until the given rescue overcomes the effect of the antifolates therapy anyhow.
Acknowledgment

This extended abstract summarizes a lecture given by G. Hempel during the 14th symposium of the “Arbeitsgemeinschaft für Pharmakologie in der Onkologie und Hämatologie” (APOH; Working Group for Pharmacology in Oncology and Hematology) held in Kiel, Germany, June 29 – 30, 2001.

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Population pharmacokinetics of oral busulfan in children

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Introduction

High-dose busulfan (bu) is an important part of many conditioning regimens before stem cell transplantation in adults and children. Several studies in children show a high inter- and intrapatient variability of oral bu disposition with eventual consequences on liver toxicity (veno-occlusive disease = VOD) and efficacy. In adults, bu dose adjustment can decrease the morbidity and mortality due to liver toxicity. So far, studies in children failed to establish a toxic level in systemic exposure (measured as area under the curve = AUC) or an applicable tool for busulfan dose adjustment. Our aim is to characterize the pharmacokinetics of oral bu in a pediatric population with the aim to build up a population-based pharmacokinetic model.

Patients, materials and methods

Plasma samples were collected from 20 children receiving busulfan orally every 6 hours over 4 days. Nineteen children received the standard dose of 4 mg/kg/d, 1 child received a dose of 5 mg/kg/d. The patient demographics were as follows (mean ± rel. SD): age 8.7 ± 4.3 years, height 1.33 ± 0.28 m, weight 33.9 ± 17.0 kg and BSA 1.1 ± 0.38 m². A total of 349 plasma samples from 147 administrations with 1 – 8 samples per administration were available (mean: 17 samples per patient over 4 days of administration). The samples were analyzed for busulfan using a LC-MS-method. Data analysis was done using NONMEM (Version V). A 1-compartment pharmacokinetic model (subroutine ADVAN2 TRANS2) and a proportional error model were applied.

Results

The best fit was obtained by inclusion of BSA as a covariate on the apparent oral clearance (Cl/F) and the apparent volume of distribution (V/F) and inclusion of interoccasion variability on clearance. Each day was defined as 1 occasion. The goodness of fit plots (Figure 1) demonstrates the high residual variability in busulfan kinetics (30%). The estimates of the pharmacokinetic parameters are summarized in Table 1. The values are comparable with those in the literature with a higher median of AUC and a smaller median of clearance. Figure 2 shows that our results do not confirm the observation that young children may have a higher apparent oral clearance of busulfan (expressed relative to BSA).

Furthermore, we found a variability of 110% for the absorption rate constant (ka) which may contribute to the high inter-individual variation in systemic exposure of bu (measured as AUC).

Figure 1. Goodness of fit plots: individual predictions vs. data.
Conclusion

Busulfan dosing calculated on the actual body weight leads to a high intra- and interindividual variability of busulfan plasma concentrations. According to our model, BSA, not body weight, is the best predictor for Cl/F and V/F. Perhaps only the new i.v. form of busulfan can avoid the problem of variable absorption of busulfan. Work is in progress to include data from other centers to get a more profound model.

Acknowledgment

This extended abstract summarizes a lecture given by G. Hempel during the symposium of the “Arbeitsgemeinschaft für Pharmakologie in der Onkologie und Hämatologie” (APOH; Working Group for Pharmacology in Oncology and Hematology) held in Köln, Germany, June 28 – 30, 2001.

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Table 1. Comparison of our results with data from pediatric patients obtained with standard 2-stage methods.

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<thead>
<tr>
<th></th>
<th>Our data</th>
<th>Vassal et al.</th>
<th>Grochow et al.</th>
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<tr>
<td></td>
<td>n = 20</td>
<td>n = 27</td>
<td>n = 30</td>
</tr>
<tr>
<td>AUC (ng/ml × h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>6,294 – 13,061</td>
<td>3,566 – 13,129</td>
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</tr>
<tr>
<td>Median</td>
<td>9,128</td>
<td>6,108</td>
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<td>V/F (l/kg)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.27 – 2.24</td>
<td>0.43 – 2.0</td>
<td>–</td>
</tr>
<tr>
<td>Median</td>
<td>0.94</td>
<td>0.81</td>
<td>–</td>
</tr>
<tr>
<td>Kₚ (1/h)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>0.14 – 9.47</td>
<td>–</td>
<td>0.11 – 5.94</td>
</tr>
<tr>
<td>Median</td>
<td>2.28</td>
<td>–</td>
<td>1.25</td>
</tr>
</tbody>
</table>

Figure 2. Relationship between busulfan apparent oral clearance (Cl/F) expressed relative to body surface area (BSA) and age.
Clinical and pharmacokinetic study with liposomal doxorubicin (DL-1) in patients with advanced metastatic cancer – results from a phase-I study

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Introduction

Liposomal encapsulation of anticancer drugs is one way to modify their pharmacokinetic behavior, thereby improving the anticancer efficacy and reducing their toxicity. The major difference between free and liposomal anticancer drugs is that not the drug, but the liposomes are first recognized by the systemic environment. Liposomal preparations of doxorubicin (DOX) have previously shown to enhance the delivery of DOX to tumor sites and clinical studies have shown improved pharmacokinetic and therapeutic indices when compared to free drug, with a notable reduction in the toxicity profile [Batist et al. 2001, Harrison et al. 1995].

DL-1 is a liposomal formulation with encapsulated doxorubicin (DOX) within membranes consisting of 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), negative charged lipids 1,2-distearoyl-sn-glycero-3-natrium phosphatic acid (DSPA) and cholesterol (5 : 1 : 4). Other liposomal formulations of DOX like Myocet and Doxil differ in their size, membrane composition, their charge and thus in the pharmacokinetic behavior. DL-1 was developed to reach high uptake rates in the liver offering a new treatment option for anthracycline-sensitive metastasis in the liver.

Material and methods

Totally, 28 patients (15 females and 13 males) with advanced cancer (different tumor types) were treated. Median age was 59 years (37 – 73). The dose was escalated from 20 (n = 3), 40 (n = 4), 60 (n = 11), 80 (n = 7) to 100 (n = 3) mg/m². DL-1 was administered as 1-hour infusion every 3 weeks. Sixteen patients, who had received 2 (or more) drug administrations, qualified for evaluation of tumor response (WHO). All patients except 1 were evaluated for toxicity (CTC). Blood samples for pharmacokinetic (PK) analysis were collected in 10 ml Na heparinate tubes following the first treatment cycle. Samples were taken prior infusion, 0.5 h during infusion, immediately after end of infusion (1 h) and 1.08, 1.17, 1.25, 1.5, 1.75, 2.0, 4.0, 6.0, 12, 24, 36 and 48 hours after the end of the infusion. The samples were processed as recently described [Maessen et al. 1988] with slight modifications. It is noteworthy to notify that the PK values reflect the total DOX in plasma, which is a mixture of encapsulated DOX plus free DOX. The pharmacokinetic parameters were determined by a 3-compartment model for the calculation of all PK values using the validated software program TopFIT version 2.0.

Results

Dose-limiting toxicities (DLT, grade 3/4) were thrombopenia, neutropenic fever, vomiting and drug-induced fever 6 – 9 hours after drug administration. DLT has been observed in 3/3 (100%) patients at the 100 mg/m² (thrombopenia, neutropenia, drug-induced fever, vomiting), in 1/7 (14%) patients at the 80 mg/m² (neutropenic fever) and in 1/11 (9%) patients at the 60 mg/m² dose level (drug-induced fever, neutropenic fever). One of 28 patients dropped out during, 8/28 after the first treatment cycle because of progressive disease and 5/28 patients dropped out because of DLT. No drug-related death and no cardiotoxicity was observed. No objective tumor response has been observed in these advanced cancer patients. However, 7/16 patients had a stable disease (SD) lasting between 9 and 18 weeks.

The PK parameters of liposomal doxorubicin (DL-1) were significantly different from that of free doxorubicin. The AUC of
corresponding dosages is much higher (10 times), the terminal half-life is shorter (0.5 times), the plasma clearance much lower (5 times) and the volume of distribution much lower (12 times) for DL-1 in comparison to DOX [Mross et al. 1988]. The plasma clearance and volume of distribution of DL-1 is more similar to TLC D-99, another liposomal formulation of DOX [Niemann et al. 2001] but differs significantly with respect to the terminal half-life. No significant metabolism was found.

**Conclusion**

The spectrum of side effects differs from that of free doxorubicin with respect to a repetitive dose-related drug-induced fever at the day of DL-1 administration. This phenomenon has been observed, too, in case of Myocet, a different liposomal DOX formulation. Neither a significant mucositis/stomatitis was observed nor acute cardiotoxicity. The possibility of cumulative chronic cardiotoxic effects as congestive heart failure (CHF) cannot be ruled out but it is still too early to draw any meaningful conclusion with respect to chronic cardiotoxicity, because all patients in the study received the drug only a few cycles, too few. Interestingly, the cardiotoxic metabolite doxorubicinol was not observed in significant amounts as it is the case of free DOX [Mross et al. 1988]. This is a hint that the encapsulated DOX DL-1 is relatively stable in plasma and the tissue uptake is much smaller in comparison to free DOX. This is reflected by the small volume of distribution at steady state. Myelotoxicity is the dose-limiting toxicity, which was expected. The pharmacokinetics of DL-1 is different from free DOX but also different to the other 2 liposomal DOX formulations. Doxil was designed as long circulating liposome because passive targeting, which is understood as passing of the liposomes through gaps in disease vasculature, and consequently higher concentrations at the tumor site, is a slow process, thus, the accumulation of liposomes at the tumor site depends besides other factors on the liposome half-life. Doxil has in comparison to free DOX and the 2 other liposomal formulations (Myocet and DL-1) a very long half-life (about 50 hours). Consequently, this drug has a very special dermatological toxicity, the hand-foot syndrome. Myocet was designed to reduce the cardiotoxicity of DOX while preserving its antitumor efficacy. This goal has been reached as was shown in a randomized study [Batist et al. 2001]. This drug has been approved in 2001 in the European Community (EU). The recommended dose for DL-1 for disease-oriented phase II trials is 80 mg/m². At this dose level of DL-1 the myelotoxicity is acceptable and similar to a DOX dose of 75 mg/m².

**Acknowledgment**

This study includes part of the thesis of T. Gierlich and was sponsored by HELM company (Hamburg). We appreciate the assistance of Dr. U. Sauer (physician), Dr. S. Fuxius (physician), Dr. U. Massing (chemist), V. Ziroli (biologist), our study nurses on the ward Hufeland, and P. Stephan (study coordination).

**References**


Oxaliplatin pharmacokinetics and pharmacodynamics in patients with metastatic colorectal cancer

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Introduction: Oxaliplatin, a novel diaminocyclohexane platinum (Pt) complex is used for the treatment of metastatic colorectal cancer in combination with fluorouracil and calcium folinate. In this study, we investigated the pharmacokinetics and the formation of Pt-DNA adducts in white blood cells as pharmacodynamic parameter after administration of oxaliplatin. The amount of adducts formed in white blood cells has been associated with tumor response after Pt-based chemotherapy [Reed et al. 1993].

Patients and methods: Patients with metastatic colorectal cancer received oxaliplatin (60 mg/m², 2-hour infusion) in combination with calcium folinate and high-dose fluorouracil in an ambulatory setting weekly. Six blood samples of each patient were analyzed for Pt in plasma and ultrafiltered plasma (representing the unbound fraction) by a validated flame less atomic absorption spectrometry (FAAS) method. Formation of Pt-DNA adducts was quantified as Pt-nucleotide ratio by UV spectrometry and FAAS [Kloft et al. 1999]. Pharmacokinetic parameters were estimated using a 1-compartment model for plasma levels and a 2-compartment model for ultrafiltrate levels.

Results: In a preliminary evaluation of 8 patients, the following pharmacokinetic parameters (means ± SD) were found (Table 1).

Conclusion: The sampling protocol with collection of only 6 samples is feasible in an ambulatory setting and allows the estimation of individual pharmacokinetic parameters of oxaliplatin. In addition, a pharmacodynamic monitoring can be performed by quantifying Pt-DNA adduct formation in white blood cells. In further investigations, interindividual differences in pharmacokinetic parameters and Pt-DNA adduct levels will be correlated to treatment-induced toxicity.

Table 1. Pharmacokinetic parameters (means ± SD).

<table>
<thead>
<tr>
<th></th>
<th>Cmax (µg/ml)</th>
<th>t1/2α (h)</th>
<th>t1/2β (h)</th>
<th>AUC (mg*h/l)</th>
<th>CL (ml/min)</th>
<th>Vss (l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>1.3 ± 0.2</td>
<td>not applicable</td>
<td>34.1 ± 10.9</td>
<td>56.6 ± 20.3</td>
<td>35.0 ± 13.3</td>
<td>96.2 ± 25.2</td>
</tr>
<tr>
<td>Ultrafiltrate</td>
<td>0.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>17.1 ± 4.3</td>
<td>3.5 ± 0.9</td>
<td>546.7 ± 136.7</td>
<td>633.9 ± 80.5</td>
</tr>
</tbody>
</table>

Pt-nucleotide ratios ranged from 2.0 to 12.8 Pt atoms per 10⁶ nucleotides increasing during 24 h after the end of oxaliplatin infusion whereas Pt levels in plasma and ultrafiltrate declined in the same time period.
in the pathogenesis of methotrexate neurotoxicity due to excitotoxic neuronal death. The determination of these amino acids in the cerebrospinal fluid (CSF) of patients receiving MTX for the treatment of cancer may elucidate their role as mediators of MTX-associated neurotoxicity. Analysis of these substances requires a highly sensitive and selective method since only trace amounts are found in CSF containing a rich variety of amino acids, bioamines, peptides and proteins. Micellar electrophoretic chromatography (MEKC) coupled with laser-induced fluorescence detection (LIF detection) might meet these requirements due to its high separation efficiency and sensitivity.

**Methods:** In this study, the suitability of 5-carboxyfluorescein succinimidyl ester (CFSE) as a fluorescent reagent to derivatize HCSA, HCA, CSA and CA was investigated. CE measurements were carried out using a P/ACE 5510 instrument (Beckman Instruments, Fullerton, CA) equipped with either a P/ACE DAD or LIF detector.

**Results:** With a buffer consisting of 0.1 M borate, 50 mM SDS and 5% (V/V) methanol (pH 9.0) baseline separation of labeled HCSA, HCA, CSA and CA was obtained. The maximum yield of derivatization reaction was obtained after 30 min using a 0.1 M borate buffer (pH 8.9) for derivatization.

Detection limits based on a signal-to-noise ratio of 3 ranged from $2.3 \times 10^{-10}$ M for HCA to $9.6 \times 10^{-10}$ M for CA, respectively. Preliminary results with cerebrospinal fluid as sample matrix revealed that the neurotransmitter aspartate (ASP) could not be separated from labeled CSA and HCA. Therefore, the separation was optimized by varying the concentration of methanol and SDS as well as by reducing the temperature. Optimum resolution was achieved performing a run in a buffer containing 0.1 M borate, 10 mM SDS and 10% (V/V) methanol (pH 9.0) at a temperature of 19 °C.

**Conclusion:** The results suggest that MEKC coupled with LIF detection is a suitable technique for the selective and sensitive analysis of excitatory amino acids in CSF. Further work will focus on the validation of the method with CSF as sample matrix.

**Development of an integrated pharmacokinetic/pharmacodynamic model for busulfan**

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Busulfan is an alkylating agent and essential part of the myeloblastic regimen to prevent marrow transplantation, used in patients suffering from hematological malignances, e.g. CML. The variability of pharmacokinetics of busulfan after oral administration is caused by multiple individual factors such as age, body weight, comedication etc. The dose-limiting toxicity is the occlusion of small central hepatic veins (veno-occlusive disease, VOD) and is thought to be related to busulfan exposition [Grochow et al. 1989]. In 1999, an intravenous form, Busulfex, has been approved by the FDA. Aims: Aim of the investigation is to characterize pharmacokinetic (PK) and pharmacodynamic (PD) of Busulfex/busulfan in order to optimize established therapy regimen based on an integrated PK/PD model. In addition, it will be evaluated whether there are (clinically) relevant differences between the i.v. and oral formulation with respect to PK and/or PD. Patients and methods: A trial with Busulfex/busulfan in patients with CML together with the Department of Hematology and Oncology, Charité, Berlin, is prepared. Blood samples will be drawn and clinical parameters will be documented in order to develop PK and PD models from the data obtained. First, a 2-hour infusion of 0.8 mg/kg BW of Busulfex will be given every 6 hours for 4 days and according to the results, the dosing regimen will be changed. The Myleran dose intended to be applied will be 1 mg/kg BW. PK/PD data analysis will be performed using the softwares WinNonlin Pro and WinNonMix Pro, Pharsight Corp., Mountain View, CA, USA. Results: Simulation studies were performed using WinNonlin to establish optimized sampling schedules. Primary steps regarding method development of an HPLC assay with fluorescence detection were successful and will be completed. Perspectives: The integrated PK/PD model will allow to describe and also predict the time course of the effect after administration of a certain dosage regimen. This will, in turn, enable the selection of the optimal dosage regimen for Busulfex/busulfan. Further contributing cooperation centers using Busulfex/Myleran in high-dose therapy prior to stem cell transplantation are highly appreciated to include patients in the trial.

**References**


**Pharmacokinetics of amifostine and its metabolites after s.c. administration**

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Amifostine (Ethyl) serves as protector against chemotherapy and radiation-induced toxicities. It is the prodrug for the dephosphorylated metabolite WR-1065, which is formed intracellularly dependent on alkaline phosphatase. Further oxidation leads to the corresponding symmetrical (WR-33278) or mixed disulfides. Determination of the prodrug amifostine as well as the resulting metabolites in plasma and urine was done after subcutaneous (s.c.) application of 500 mg amifostine in patients with head and neck cancer. Amifostin was given daily, 5 times a week, for 6 weeks prior to radiotherapy. To clarify the question of a possible non-linear pharmacokinetic behavior of amifostine, plasma and urine analysis was performed on days 1, 4, and 5 of week 1, and for the first days of the following weeks of treatment. For the quantification of amifostine, WR-1065 and the disulfides a RP-HPLC method was performed, using a solvent system consisting of 18% acetonitrile and 82% aqua (20 mM dipotassium hydrogen phosphate, 5 mM octyl sulfate, 1 mM TBHAS, pH = 3.00 with orthophosphoric acid), the detection was done electrochemically with a Pt working elec-
trode and an Ag/AgCl counter electrode using a potential of +1.00 V. The limit of quantification was 10 ng/ml WR-1065. The within- and between-day accuracy of the assay for WR-1065 varied from 5% to 15% (from 100 µg/ml to 10 ng/ml, respectively), the precision was in the range of 8%. Amifostine was converted to WR-1065 by acidic hydrolysis at 42 °C for 12 h, the disulfides were reduced to WR-1065 by the use of DTT within 15 min at RT. Calculation of amifostine and the disulfides was done after conversion, by subtracting the measured concentrations of the active metabolite WR-1065. Up to now, 5 patients were enrolled into this study. There is evidence for an accumulation of the active metabolite WR-1065 in plasma, leading to an increase of Cmax as well as AUC after repeated s.c. administrations. The mean terminal half-life for the parent compound was calculated in a 1-compartmental analysis to 40 min, for WR-1065 to 60 min. Tmax for amifostine and WR-1065 was in the range of 50 min after application, whereas the peak plasma level for the di-thiols was observed after 70 min. The disulfides were cleared much slower from the plasma than amifostine and WR-1065, leading to an increase of the peak plasma levels of WR-1065. This indicates a saturation of the disulfide formation or changes in the uptake of tissues or the elimination of WR-1065.

Plasma as well as cellular pharmacokinetics of doxorubicin after application of pegylated liposomes (caelyx): A new option in the treatment of leukemia?


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Caelyx (doxil) is a doxorubicin-encapsulated formulation of polyethylene glycol-coated liposomes with a long terminal half-life (t1/2) and a minimal volume of distribution in steady state (Vss). It has demonstrated activity against solid tumors with mild myelosuppression, minimal hair loss and a low risk of cardiotoxicity. For this new form of doxorubicin, a better pharmacokinetic profile than for the administration of free doxorubicin was obtained. It was the aim of this study to analyze the plasma levels as well as the cellular uptake of encapsulated and free doxorubicin. For this purpose, caelyx was administered i.v. to patients with various refractory malignancies up to doses of 60 mg/m² (doxorubicin HCl equivalent) within 1 hour, once every 28 days, for as long as patients respond to and tolerate treatment. Pharmacokinetic analysis of caelyx as well as for the released doxorubicin were done in plasma, urine and intracellular. Plasma and cell samples were taken on day 1, 2, 3, 4, 5, 8, 15, 22 and 28 of each cycle, urinary excretion was analyzed within the first 48 h after dosing. For the quantification of caelyx, doxorubicin and doxorubicinol, a validated RP-HPLC method was used. Separation of encapsulated doxorubicin from free doxorubicin levels was performed by the use of a silica absorption method. Quantification of caelyx was done as free doxorubicin after destruction of the liposomes using HCl/methanol. The limit of detection for doxorubicin and its metabolites was 0.1 ng/ml. The intra- and inter-day accuracy for this assay varied from 5% to 15% (from 10 µg/ml to 0.1 ng/ml, respectively), the precision was in the range of 7%. Up to now, blood and urine samples (n = 360 plasma- and n = 300 urine samples) of 19 patients were enrolled into this study. The obtained accumulation of the liposomal as well as the free doxorubicin in plasma was only marginal in this setting. The mean t1/2 for caelyx was calculated to 2.5 days, total clearance (Cltot) was determined to less than 1 ml/min. There was no renal excretion (Clen) of the intact liposomes detectable. The mean t1/2 for released doxorubicin was calculated to about 3 days, Cltot to 180 ml/min, with a renal excretion of about 1% within the first 48 h. The mean area under concentration vs. time curve (AUC) was calculated to 260 ng/ml × d, which is at least 3-fold higher than after conventional application of the equivalent dose (60 mg/m²) of free doxorubicin. The mean peak plasma concentration (Cmax) of free doxorubicin after a 1-hour infusion of 60 mg/m² caelyx was calculated to 50 ng/ml, which is only 1/20 compared to the equivalent conventional dosing. Cellular uptake of doxorubicin increased after application of caelyx, compared to the intra-individual uptake of the equivalent conventional dose of doxorubicin. Therefore, beside the indirect tumor targeting using the long circulating pegylated liposomes, the slow-release of doxorubicin enables the treatment of possible (micro) metastases and furthermore offers an option in the management of leukemia.