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ORIGINAL ARTICLE

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Bioequivalence investigation of high-dose etoposide and etoposide phosphate in lymphoma patients

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Abstract *Purpose*: To compare etoposide pharmacokinetics following administration of high-dose etoposide and etoposide phosphate, a water-soluble prodrug of etoposide. Bioequivalence was assessed using a two-treatment randomized crossover design. *Methods*: Ten patients with high-risk or relapsed lymphoma were treated with a sequential high-dose chemotherapy. They were randomized to receive either 3×400 mg/m^2 etoposide or an equimolar amount of etoposide phosphate (as 1-h infusions on three consecutive days) in the first course and the alternative drug in the second course. Serial plasma and ultrafiltered plasma samples were collected and analysed for etoposide by a reversed-phase HPLC method with UV and electrochemical detection. Pharmacokinetic parameters were estimated using a two-compartment model. Bioequivalence was assessed calculating the 90% confidence intervals (CI) for the ratios of the geometric means of $AUC_{0-\infty}$ and additionally of C_{max} of etoposide derived from etoposide phosphate relative to etoposide in plasma and ultrafiltered plasma as point estimates (level of significance $\alpha < 0.05$). *Results*: Pharmacokinetic parameters of etoposide were comparable in both treatment arms except that terminal half-life was significantly shorter and apparent V_{ss} in ultrafiltered plasma was significantly larger following administration of the prodrug. The point estimates for $AUC_{0-\infty}$ of etoposide derived from etoposide phosphate relative to etoposide were 102.9% and 88.4% for plasma and ultrafiltered plasma, respectively. The 90% CIs were in the range from 80% to 125% where bioequivalence can be assumed. The point estimates of C_{max} on day 3 of chemotherapy were 96.5% and 81.7% in plasma and ultrafiltrate with the 90% CI in ultrafiltered plasma being out of the range from 80% to 125%. *Conclusion*: With respect to total drug exposure, represented by $AUC_{0-\infty}$, high-dose etoposide phosphate is bioequivalent to high-dose etoposide.

Keywords Etoposide · Etoposide phosphate · Pharmacokinetics · High-dose chemotherapy · Bioequivalence

Abbreviations $AUC_{0-\infty}$: area under the concentration-time curve extrapolated to infinity · *CI*: confidence interval · *CL*: total body clearance · C_{max}: maximum concentration · *CV*: coefficient of variation · *F*: fraction of etoposide phosphate converted to etoposide · $t_{1/2\alpha}$ $t_{1/2\beta}$: disposition half-lives · t_{max}: time to achieve maximum concentration · V_{ss}: volume of distribution at steady-state

Introduction

Etoposide is a semisynthetic derivative of podophyllotoxin which is active against a variety of different tumours [15]. It is used in standard-dose as well as in high-dose chemotherapy regimens [16]. During the past 10 years, high-dose etoposide with stem cell support has been introduced into therapy of malignancies such as Hodgkin's disease and non-Hodgkin's lymphoma, germ cell tumour, and small-cell lung cancer [1, 7, 19, 25]. However, administration of high-dose etoposide has a number of limitations. Since aqueous solubility of etoposide is very poor, etoposide for intravenous use is formulated in ethanol, polysorbate 80, benzyl alcohol, and polyethylene glycol. In order to avoid precipitation,

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etoposide has to be diluted to a final maximum concentration of 0.4 mg/ml in aqueous solution resulting in large fluid volumes in high-dose treatment. Therefore, high-dose etoposide is usually administered as undiluted solution for intravenous injection [9, 20] which has been shown to be associated with the risk of damage to polyurethane catheters during continuous infusion [34]. In addition, hypotension and allergic reactions have been observed in patients treated with high-dose etoposide [6]. Hypersensitivity reactions have been suggested to be at least in part related to the solubilizers in the etoposide formulation [14].

Etoposide phosphate is a water-soluble prodrug of etoposide that is rapidly converted to the active compound, presumably by endogenous phosphatases [24]. No saturation of metabolic activation occurs even with intravenous bolus administration of etoposide phosphate at standard therapeutic dosages [3]. Pharmacokinetic studies with etoposide phosphate have shown pharmacokinetics comparable to those of etoposide after administration of the prodrug [2, 11, 31]. Bioequivalence has been shown for standard-dose therapy [17, 21]. In high-dose chemotherapy, phase I studies have demonstrated linear etoposide pharmacokinetics up to etoposide phosphate doses equivalent to 1200 mg/m^2 etoposide given as a 2-h intravenous infusion [18].

No intraindividual comparison of etoposide pharmacokinetics following administration of high-dose etoposide and etoposide phosphate to assess bioequivalence has yet been performed. The etoposide-containing sequential high-dose chemotherapy regimen, implemented in two clinical trials for lymphoma, offered the possibility of conducting an intraindividual comparative study of etoposide and etoposide phosphate. Since etoposide is extensively bound to plasma proteins [26], bioequivalence was investigated in plasma as well as in ultrafiltered plasma. In the study reported here we compared the pharmacokinetic parameters of etoposide intraindividually following treatment with equivalent doses of the two compounds in a sequential high-dose chemotherapy regimen.

Materials and methods

Patients and treatment

Patients with high-risk or relapsed lymphoma were treated with two courses of high-dose chemotherapy followed by peripheral blood stem cell transplantation. The chemotherapy regimen consisted of etoposide, ifosfamide, carboplatin, doxorubicin, and dexamethasone. Ten patients (median age 47 years, range 21 to 62 years) participated in this "two-treatment, two-period, two-sequence (2×2) randomized crossover study". The study protocol was approved by the Ethical Committee of the Charité Virchow Hospital. All patients gave written informed consent before entering the study.

Patients were randomized to receive either 3×400 mg/m^2 etoposide (Vepesid, Bristol-Myers Squibb, Munich, Germany) or an equimolar amount of etoposide phosphate (Etopophos, Bristol-Myers Squibb) as 1-h intravenous infusions on three consecutive days in the first course and the alternative drug in the second course after 21 to 28 days. Etoposide was administered as undiluted solution for injection whereas etoposide phosphate was diluted in water for injection to a final concentration of 10 mg/ml.

Serial blood samples were drawn before and at the end of the first and second intravenous infusion as well as before and 0, 1, 3, 8, 20, 30, and 44 h after the third infusion. Blood samples were collected from a central venous catheter into tubes (Monovetten; Sarstedt, Nuembrecht, Germany) containing potassium EDTA (1.2–2 mg EDTA/ml) to prevent further conversion of etoposide phosphate to etoposide [3]. Plasma was separated by centrifugation at 3200 *g* for 5 min. To determine the concentration of etoposide not bound to macromolecules, 1 ml of each plasma sample was centrifuged at 2000 *g* for 20 min through an ultrafiltration membrane with a cut-off of 30 kDa (Centrifree; Millipore, Eschborn, Germany). Plasma and ultrafiltered plasma (ultrafiltrate) samples were stored at –70°C until analysis.

Sample preparation and etoposide analysis

Plasma and ultrafiltered plasma samples were analysed for etoposide by a modified reversed-phase HPLC method [10, 29]. In brief, a Hypersil ODS RP-18 column (Knauer, Berlin, Germany) was used as stationary phase. To analyse plasma samples, the mobile phase consisted of acetonitrile/methanol/0.01 *M* Na_2HPO_4 (4.5/35.0/60.5 v/v) and was adjusted with H_3PO_4 to pH 5.3. The flow rate was set at 0.4 ml/min. A 100 µl aliquot of plasma was mixed with 20 µ*L* of a 1 mg/ml solution of ascorbic acid. Then, 250 µl acetonitrile were added to precipitate plasma proteins. After centrifugation at 3200 *g* for 10 min, the supernatant was evaporated to dryness. The residue was dissolved in 100 µl mobile phase and 40 µl were injected onto the HPLC system. Etoposide was quantified using UV detection at 210 nm. The limit of quantification was 0.092 µg/ml and calibration curves were linear with $R^2 \geq 0.999$ up to 200 µg/ml. Within-day precision was 3.1% (etoposide concentration 1.0 µg/ml, $n = 10$), between-day precision was 4.3% (etoposide concentration 57.1 µg/ml, $n = 11$) and 4.5% (etoposide concentration 0.26 µg/ml, $n = 11$). Accuracy was determined at the same etoposide concentrations as the between-day precision and was –2.7% and –0.15%, respectively ($n = 11$).

The mobile phase to analyse ultrafiltered plasma samples consisted of methanol/0.01 *M* Na_2HPO_4 (45/55 v/v) and was adjusted with H_3PO_4 to pH 6.0. The flow rate was set at 0.4 ml/min. Ultrafiltered plasma samples (50 µl) were diluted with 25 µl 0.02 *M* Na_2HPO_4 (pH 5.3) to prevent etoposide degradation during sample processing and analysis. A 40-µl aliquot of the prepared sample was injected onto the HPLC system. Because of the low unbound concentrations expected, etoposide was quantified in ultrafiltered plasma by electrochemical detection which was shown to be more sensitive than UV detection. The potentials of the dual electrode cell were set at 100 and 500 mV. The limit of quantification was 0.010 µg/ml and calibration curves were linear with $R^2 \geq 0.98$ up to 9.45 µg/ml. Within-day precision was 4.2% (etoposide concentration 2.6 µg/ml, $n = 10$), between-day precision was 10.2% (etoposide concentration 0.87 µg/ml, $n = 9$) and 8.1% (etoposide concentration 1.89 µg/ml, $n = 9$). Accuracy was determined at the same etoposide concentrations as the between-day precision and was –10.5% and –1.2%, respectively ($n = 9$).

Pharmacokinetic data analysis

A compartmental approach was used for pharmacokinetic data analysis. A two-compartment model with zero-order input and first-order elimination from the central compartment was applied for parameter estimation using the software program SipharWin (release 1.14; Simed, Créteil, France). In a first step, initial parameter estimation was performed on the etoposide plasma and ultrafiltrate concentrations after the third infusion using the automatic peeling algorithm [12]. In a second step, the model was fitted

to experimental data applying a numerical algorithm based on the Powell method to minimize the objective function [23] using the weighting factor $1/y^2$.

The terminal half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$), the area under the concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$) and the volume of distribution at steady-state (V_{ss}) were estimated from the primary model parameters. The percentage of the AUC extrapolated was less than 15%. Total body clearance (CL) of etoposide was calculated by dividing the dose administered by the observed $AUC_{0-\infty}$. As the percentage of etoposide phosphate converted into etoposide (available fraction F) is unknown, the apparent volume of distribution at steady-state (V_{ss}/F) and apparent total body clearance (CL/F) of etoposide following administration of the prodrug were estimated.

Statistical analysis and bioequivalence criteria

Descriptive statistics comprised the calculation of arithmetic means and standard deviations.

Regulatory agencies recommend parametric general linear model procedures for the analysis of pharmacokinetic data derived from in vivo bioequivalence studies [4, 8]. The pharmacokinetic parameter $AUC_{0-\infty}$ as a measure of total systemic exposure is considered the most important criterion for bioequivalence assessment [5]. In addition, C_{max} was evaluated as a measure of peak exposure. After natural logarithmic transformation, an analysis of variance (ANOVA) was performed on $AUC_{0-\infty}$ and C_{max} using the software program SPSS (SPSS for Windows release 7.5; SPSS Software, Munich, Germany). Following the FDA guidelines for bioequivalence studies, the statistical model applied contained the factors "treatment" (two levels), "period" (two levels), "subject" (ten levels) nested in "sequence" (two levels).

As point estimates (i.e. single estimates of the unknown population mean) for bioavailability of etoposide derived from etoposide phosphate (test, T) relative to etoposide (reference, R) the ratios of the geometric means of the pharmacokinetic parameters $AUC_{0-\infty}$ and C_{max} were calculated. Constructing the 90% confidence interval for the ratio between the test and the reference means of $AUC_{0-\infty}$ and C_{max} is equivalent to carrying out two one-sided tests of hypothesis at the $\alpha = 0.05$ level of significance. Bioequivalence was assumed if the 90% confidence interval (CI) for the T/R ratio fell in the range from 80% to 125% for the log-transformed data.

For additional information, the point estimates as well as the 90% CI of half-lives ($t_{1/2\alpha}$ and $t_{1/2\beta}$), CL and V_{ss} were estimated as described previously. A non-parametric test (Wilcoxon signed rank test) for related samples was used to compare the fraction not bound to plasma proteins between treatments and to compare maximum etoposide concentrations in plasma and ultrafiltered plasma samples both within and between treatments. The level of significance was set to $\alpha < 0.05$. The root mean square error calculated from the ANOVA residual error for each pharmacokinetic parameter was used to estimate intraindividual variability expressed as coefficient of variation (CV).

Results

Plasma concentration-time profiles

Peak and trough concentrations of etoposide were determined on days 1 and 2 of treatment, whereas serial etoposide concentrations were evaluated after the third infusion. Mean plasma and ultrafiltrate concentration-time curves of etoposide following intravenous infusion of etoposide and etoposide phosphate equivalent to a dose of 3×400 mg/m^2 etoposide are shown in Figs. 1 and 2, respectively.

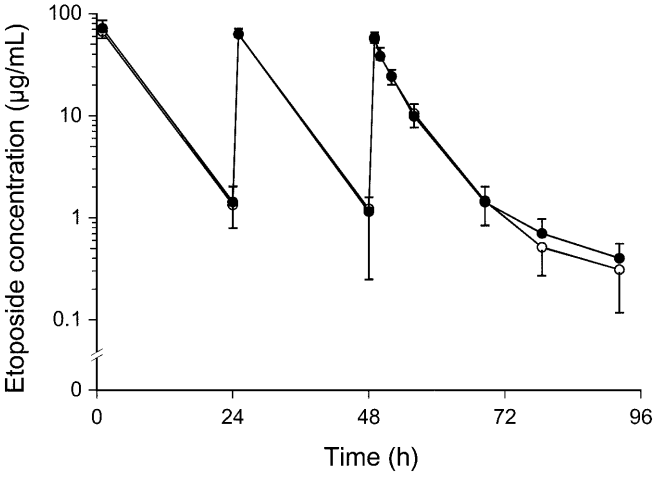

Fig. 1 Plasma concentration-time profiles (means ± SD) of etoposide following administration of 3×400 mg/m^2 etoposide (●) and etoposide phosphate (○) ($n = 10$)

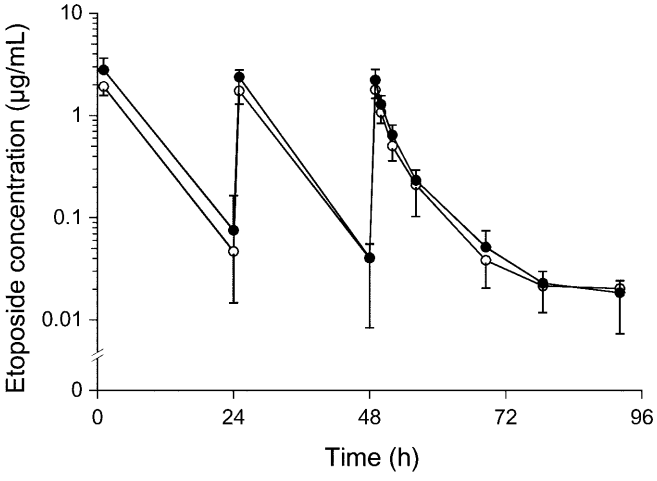

Fig. 2 Ultrafiltrate concentration-time profiles (means ± SD) of etoposide following administration of 3×400 mg/m^2 etoposide (●) and etoposide phosphate (○) ($n = 10$)

Pharmacokinetic parameters

Mean pharmacokinetic parameters of etoposide and the estimates for bioequivalence assessment are summarized in Table 1 (plasma) and Table 2 (ultrafiltrate). No significant differences in the pharmacokinetic parameters $t_{1/2\alpha}$, $AUC_{0-\infty}$ and CL between the two treatment arms in either plasma or ultrafiltrate could be detected by ANOVA. Following treatment with etoposide phosphate, mean $t_{1/2\beta}$ of etoposide was significantly shorter in both plasma and ultrafiltrate compared to the treatment with etoposide. Apparent V_{ss} of etoposide after treatment with the prodrug was significantly larger than V_{ss} after etoposide in ultrafiltered plasma but not in plasma. No significant differences could be observed in the mean fraction of etoposide not bound to macromolecules. Following treatment with etoposide and etoposide phosphate, mean values of the unbound

Table 1 Pharmacokinetic parameters of etoposide (means ± SD) and bioequivalence assessment of etoposide following administration of 400 mg/m^2 etoposide and etoposide phosphate in plasma ($n = 10$) (*CI* confidence interval, *CV* coefficient of variation)

	AUC (μg·h/ml)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	CL or CL/F[a] (ml/min)	V_{ss} or V_{ss}/F[a] (l)
Etoposide administered	290.7 ± 35.0	2.3 ± 0.3	12.3 ± 3.8	42.4 ± 4.8	16.3 ± 3.2
Etoposide phosphate administered	300.3 ± 45.9	2.3 ± 0.5	10.3 ± 3.1*	41.2 ± 5.4	14.6 ± 2.3
Point estimate (%)	102.9	100.2	82.8	96.8	90.4
90% CI upper limit (%)	108.1	117.6	102.0	102.5	97.2
90% CI lower limit (%)	97.7	82.8	63.5	91.2	83.7
Intraindividual CV (%)	6.3	20.9	23.1	6.8	8.1

*Significant difference (CI not in the range 80–125%)
[a]For etoposide phosphate

Table 2 Pharmacokinetic parameters of etoposide (means ± SD) and bioequivalence assessment of etoposide following administration of 400 mg/m^2 etoposide and etoposide phosphate in ultrafiltered plasma ($n = 10$) (*CI* confidence interval, *CV* coefficient of variation)

	AUC (μg·h/ml)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	CL or CL/F[a] (ml/min)	V_{ss} or V_{ss}/F[a] (l)
Etoposide administered	9.2 ± 1.6	1.7 ± 0.2	14.4 ± 5.0	1356 ± 218	547 ± 102
Etoposide phosphate administered	8.2 ± 1.5	1.8 ± 0.3	12.7 ± 2.2*	1528 ± 229	630 ± 76*
Point estimate (%)	88.4	109.0	90.4	112.7	116.3
90% CI upper limit (%)	94.1	120.6	103.8	118.16	126.2
90% CI lower limit (%)	82.7	97.4	77.0	107.4	106.5
Intraindividual CV (%)	6.9	14.0	16.1	6.4	11.8

*Significant difference (CI not in the range 80–125%)
[a]For etoposide phosphate

Table 3 Maximum plasma concentrations of etoposide (μg/ml, means ± SD) and bioequivalence assessment of etoposide following administration of 400 mg/m^2 of etoposide and etoposide phosphate as 1-h intravenous infusion on three consecutive days ($n = 10$) (*CI* confidence interval, *CV* coefficient of variation)

	Day 1	Day 2	Day 3
Etoposide administered	72.0 ± 13.8	63.1 ± 8.0	58.7 ± 6.9
Etoposide phosphate administered	66.7 ± 9.2	62.9 ± 4.2	56.4 ± 4.7
Point estimate (%)	94.4	100.2	96.5
90% CI upper limit (%)	108.1	106.9	102.9
90% CI lower limit (%)	80.7	93.5	90.0
Intraindividual CV (%)	15.4	8.0	7.7

Table 4 Maximum ultrafiltrate concentrations of etoposide (μg/ml, means ± SD) and bioequivalence assessment of etoposide following administration of 400 mg/m^2 of etoposide and etoposide phosphate as 1-h intravenous infusion on three consecutive days ($n = 10$) (*CI* confidence interval, *CV* coefficient of variation)

	Day 1	Day 2	Day 3
Etoposide administered	2.80 ± 0.86	2.38 ± 0.41	2.23 ± 0.62
Etoposide phosphate administered	1.92 ± 0.35*	1.74 ± 0.45*	1.79 ± 0.31*
Point estimate (%)	73.8	70.4	81.7
90% CI upper limit (%)	91.0	90.0	99.8
90% CI lower limit (%)	56.6	50.8	63.6
Intraindividual CV (%)	17.7	22.0	21.7

*Significant difference (CI not in the range of 80–125%)

fraction were 3.8 ± 0.7% and 3.2 ± 0.4% at the end of infusion and 3.6 ± 1.2% and 4.5 ± 0.5% 28–32 h after the end of infusion, respectively. The unbound fraction was not concentration-dependent over the range observed.

Bioequivalence assessment

In order to assess bioequivalence, the parameters $AUC_{0-\infty}$ and C_{max} are described in more detail. Maximum plasma concentrations were reached at the end of intravenous infusion in both treatment arms. Mean plasma etoposide C_{max} values following administration of etoposide and etoposide phosphate are listed in Table 3. In both treatment arms, mean maximum plasma concentrations were significantly lower after the third compared to those after the first drug administration. The point estimates for the bioequivalence assessment of etoposide derived from etoposide phosphate relative to etoposide were 102.9% (90% CI 97.7% to 108.1%) based on $AUC_{0-\infty}$ and 96.5% (90.0% to 102.9%) based on C_{max} after the third drug administration.

In ultrafiltered plasma, the results were slightly different. Mean C_{max} values of etoposide not bound to macromolecules after intravenous infusion of etoposide and etoposide phosphate are listed in Table 4. In ultrafiltrate, no significant differences between mean C_{max} of etoposide after the first and the third intravenous infusion could be detected in either treatment arm. Mean C_{max} values of etoposide in ultrafiltered plasma were significantly lower on all days of treatment after etoposide phosphate compared to the respective C_{max} after treatment with etoposide. In ultrafiltrate, the point estimates for the bioequivalence assessment of etoposide derived from etoposide phosphate relative to etoposide were 88.4% (82.7% to 94.1%) based on $AUC_{0-\infty}$ and

81.7% (63.6% to 99.8%) based on C_{max} after the third drug administration.

Discussion

An intraindividual comparative crossover study was conducted to assess bioequivalence of high-dose etoposide and etoposide phosphate. Since etoposide is highly bound to plasma proteins, bioequivalence of high-dose etoposide and etoposide phosphate was also assessed with regard to the pharmacodynamically active fraction of etoposide. In our study, the fraction of etoposide not bound to macromolecules (3.8% and 3.2% at the end of infusion) was comparable to the findings of Stewart et al. [26] who reported a mean unbound fraction of $4.3 \pm 0.4\%$ in plasma from healthy volunteers. In contrast, the mean unbound fraction was found to be increased with a higher variability in cancer patients with hypoalbuminaemia [22] and hyperbilirubinaemia [26] compared to healthy volunteers. Since patients with hepatic dysfunction defined as serum bilirubin levels > 1.5 mg/dl were not included in this study, our results might rather be comparable to those reported in healthy volunteers than reported in cancer patients.

Pharmacokinetic parameters obtained in this study were comparable to those reported by others. However, the terminal half-life of etoposide was rather long with mean values of 12.3 and 14.4 h in plasma and ultrafiltered plasma, respectively, compared to reported mean values in the range 4–8 h [13]. The use of a sensitive etoposide assay and a relatively long sampling period might have contributed to this difference in terminal half-life compared to those reported by others.

Statistically significant differences in etoposide pharmacokinetics between both treatment arms were observed for terminal half-life and V_{ss}. Mean $t_{1/2\beta}$ was significantly shorter following administration of etoposide phosphate in both plasma and ultrafiltered plasma samples. Since only the apparent terminal phase of the concentration-time curve was different, as can be seen in Fig. 1, the difference in $t_{1/2\beta}$ was not likely to be related to the conversion of etoposide phosphate into etoposide. Therefore, the longer $t_{1/2\beta}$ of etoposide in plasma after treatment with the etoposide formulation probably resulted from altered distribution which was also indicated by a slightly smaller mean apparent V_{ss} of etoposide in plasma given as etoposide phosphate. A possible explanation might be the addition of solubilizers in the etoposide formulation. Polysorbate 80, a non-ionic detergent in the etoposide intravenous formulation, is known to affect biological membranes [30]. Furthermore, polysorbate 80 has been reported to increase the accumulation of etoposide in different human lung carcinoma cells in vitro in a dose-dependent manner [32]. As the amount of polysorbate 80 in the solution for injection is four times the amount of etoposide, considerable concentrations of polysorbate 80 might be achieved at the end of intravenous infusion. Therefore, an alteration in etoposide distribution leading to an increase in terminal half-life of etoposide after treatment with etoposide solution for injection might be produced by altered membrane passage into tumour and/or normal cells.

In contrast, mean apparent V_{ss} of etoposide in ultrafiltered plasma samples was significantly larger following administration of the prodrug. As etoposide phosphate is a prodrug of etoposide, the apparent total body clearance (CL/F) and the apparent volume of distribution (V_{ss}/F) were estimated following treatment with etoposide phosphate with F affected by the conversion of the prodrug into the active compound. Incomplete conversion of the prodrug would result in a larger mean apparent V_{ss}. Moreover, further mechanisms might also have an impact on estimation of pharmacokinetic parameters in ultrafiltrate. In addition to the conversion of the prodrug by endogenous phosphatases, plasma protein binding including adsorption and desorption processes of both etoposide phosphate and the already converted etoposide contribute to the observed etoposide concentrations in ultrafiltered plasma.

Peak etoposide concentrations were determined at the end of intravenous infusion of etoposide and etoposide phosphate on three consecutive days. In plasma, a significant decrease in C_{max} was observed from day 1 to day 3 of chemotherapy following administration of both compounds. As the decrease in maximum plasma concentrations was comparable in the two treatment arms, the effect was unlikely to have been related to treatment. One possible explanation could be a higher rate of metabolism. Ifosfamide, which was part of the combination chemotherapy regimen, is a known substrate of cytochrome P450 (CYP) 3A [33] with the property of autoinduction of metabolism. In germ cell tumour patients receiving etoposide and ifosfamide as part of the CEI chemotherapy regimen, increasing concentrations of etoposide catechol, an etoposide metabolite formed by CYP3A4 in the liver, were determined over 4 days [28]. Increasing concentrations of etoposide catechol from day 1 to day 3 were also observed in this study (data not shown) corresponding to decreasing C_{max} of etoposide at the end of intravenous infusion in plasma. However, only a slight tendency towards decreasing C_{max} of etoposide could be detected in ultrafiltrate.

Bioequivalence was primarily assessed by $AUC_{0-\infty}$. Based on this parameter, bioequivalence of etoposide and etoposide phosphate can be assumed. The 90% CI was in the range 80–125% in both plasma and ultrafiltered plasma although the point estimate of $AUC_{0-\infty}$ in ultrafiltered plasma was only 88.4%. In addition, C_{max} as a measure of peak exposure was evaluated using the same methodology. In plasma the 90% CI of the ratio of C_{max} of etoposide after treatment with etoposide phosphate relative to C_{max} of etoposide was in the acceptance range. This indicates that no saturation of dephosphorylation occurred, even when higher doses of etoposide phosphate were administered. In contrast, C_{max} of etoposide in ultrafiltered plasma was significantly lower

following administration of etoposide phosphate compared to administration of etoposide. The ratio of C_{max} of etoposide derived from etoposide phosphate relative to C_{max} of etoposide did not fall in the range 80–125% suggesting a non-equivalent peak exposure to unbound etoposide. The contradictory findings in plasma and ultrafiltered plasma might again be related to the fact that etoposide has to be generated from the prodrug after treatment with etoposide phosphate. It is likely that after treatment with etoposide phosphate, the ''true'' maximum etoposide concentrations were achieved after a short delay following the end of intravenous infusion where no concentration-time points were available.

For the interpretation of the results it has to be considered that there is some evidence that AUC determines toxicity and probably efficacy of etoposide rather than a single time-point such as C_{max}. Haematotoxicity as a surrogate pharmacodynamic parameter has been found to correlate with exposure to etoposide in both plasma and ultrafiltered plasma [27]. Therefore, and because of the probable underestimation of the ''true'' C_{max} after administration of etoposide phosphate, $AUC_{0\text{-}\infty}$ of etoposide is regarded as the relevant parameter for the bioequivalence decision.

In conclusion, with respect to total drug exposure high-dose etoposide phosphate can be considered bioequivalent to high-dose etoposide in both plasma and ultrafiltered plasma. From our pharmacokinetic results, no differences in antitumour activity of etoposide derived from high-dose etoposide phosphate should be expected. More detailed information on the pharmacokinetics of etoposide phosphate is needed to explain the differences in maximum unbound etoposide concentrations at the end of intravenous infusion of etoposide phosphate.

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