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Effects of verapamil on the pharmacokinetics and metabolism of epirubicin*

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Summary. Experimental data suggest that multidrug resistance in cancer may be overcome by using an increased dose of anticancer agent(s) in combination with a resistance-modifying agent (RMA). We studied the pharmacokinetics and metabolism of both epirubicin (EPI) and verapamil (VPL) to explore the possible pharmacokinetic interactions between these two drugs. Ten patients with advanced breast cancer were given EPI (40 mg/m^2 in a daily i.v. bolus for 3 consecutive days), and five of them also received VPL (4 × 120 mg/daily p.o. for 4 consecutive days). The data indicated a significant interaction between these two drugs that affected their metabolism. The areas under the concentration-time curves (AUC) obtained for epirubicin glucuronide, epirubicinol glucuronide, and both of the 7-deoxy-aglycones were higher in the EPI + VPL group as compared with the EPI group. The AUC, terminal half-life, mean residence time, volume of distribution at steady state, and plasma clearance of EPI alone as compared with EPI + VPL did not differ significantly. These results suggest either an induction of enzymes necessary for drug metabolism or an increase in the liver blood flow, resulting in an enhanced generation of metabolites with time or in an inhibition of excretion processes. Comparisons of the AUC values obtained for EPI and its metabolites after the first, second, and third injections of EPI revealed a cumulative effect for the metabolites that was more pronounced in the EPI + VPL group, being significant ($P < 0.05$) for epirubicin glucuronide in both treatment groups and for epirubicinol glucuronide in the EPI + VPL group. Maximal concentrations of VPL and nor-VPL reached 705 ± 473 and 308 ± 122 ng/ml, respectively, with the steady-state concentrations being 265 ± 42 ng/ml for VPL and 180 ± 12 ng/ml for nor-VPL.

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Introduction

Verapamil (VPL) can enhance the efficacy of anthracyclines in vitro [12] and in vivo [11, 16]. Because intrinsic and aquired drug resistance remains a major problem in cancer chemotherapy, especially in metastatic disease, great interest and enthusiasm has been generated by studies showing that a variety of agents can be successfully used to overcome resistance in vitro [30]. VPL represents a prototype multidrug resistance (MDR)-modifying agent [32] that has been used in several clinical studies [7, 21, 26]. A quantitative relationship has been established between the reversal of MDR by resistance-modifying agents (RMAs) and a concomitant decrease in intracellular levels of doxorubicin in MDR cells. A decrease in drug accumulation and a change in intracellular drug disposition are the determinants of anthracycline resistance in MDR cells [29]. The amount of drug necessary to reverse resistance depends on the degree of resistance. From in vitro studies it is known that the higher the concentration, the better; in cell-culture systems the best results were obtained at VPL concentrations ranging from 2.5 to at least 8 μM [13], but the latter cannot be reached in humans because of cardiac toxicity.

In contrast to other studies in which VPL has been given i.v. as a continuous infusion in an intensive care unit (ICU), in the present trial we investigated the high-dose administration of VPL via the oval route, as the plasma concentrations required for a given increase in atrioventricular (AV) conduction time after oral dosing appear to be 2- to 3-fold those required following i.v. administration to the same subject [14]. This difference has been attributed to presystemic stereoselective elimination of the more active L-isomer of VPL. Drug levels of 600–1,300 ng/ml (1.2–2.6 μM) have been achieved following oral administration as well as after continuous infusions [7, 21, 26]. Drug-resistance modulation can also be expected at such lower concentrations, since there is a linear relationship between the resistance factor and the amount of VPL necessary to reverse resistance [29]. Epirubicin (EPI) undergoes extensive hepatic metabolism and biliary excretion [3, 34]. VPL has vasodilator properties and has been

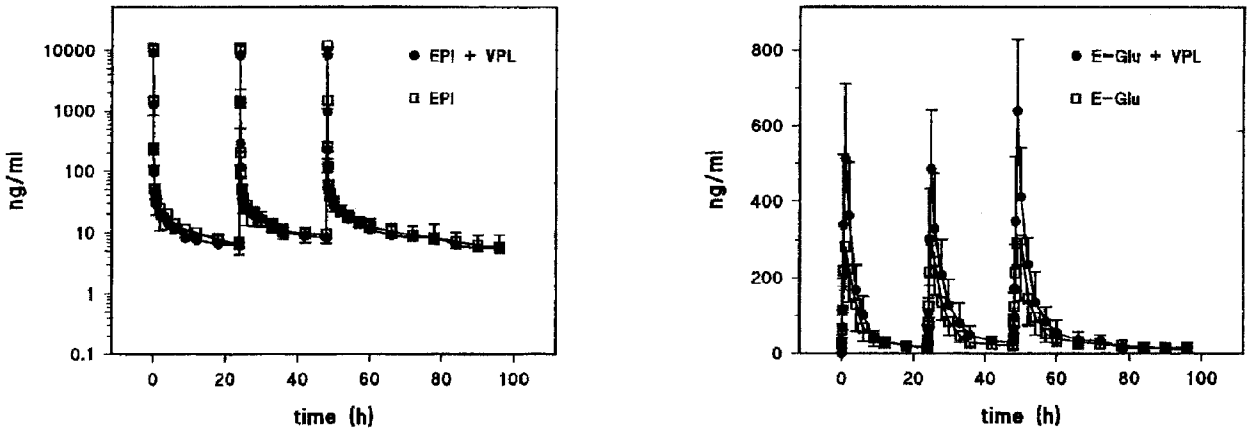

Fig. 1. C(t) curves obtained for EPI and E-Glu during treatment of patients with EPI alone or in combination with VPL. Data represent mean values ± SD; differences are not statistically significant

shown to increase the liver blood flow [20]. A randomized clinical phase II study using EPI alone and in combination with VPL was performed in patients with metastatic breast cancer because of recent evidence that the incidence of P-glycoprotein overexpression in primary breast-cancer specimens can be high (56%) [15], an assumption that served as the basis for the clinical study [25]. The pharmacokinetics of VPL and of intensive-dose EPI (given on a 3-day split-dose regimen), including the metabolism of the two drugs, was studied.

Patients and methods

Ten patients with histologically confirmed advanced breast cancer were studied. Adjuvant chemotherapy containing no anthracycline as well as pretreatment with hormones and/or radiotherapy were allowed. During the pharmacokinetics study, no medication other than the study drugs was given. Liver and kidney function as well as morphology were normal. EPI was given via a central line as an i. v. bolus injection (1–2 min) at 40 mg/m^2 daily on days 1–3, and VPL was given orally at 4 × 120 mg daily on days 0–3. VPL administration was started on day 0 to enable an investigation of the side effects of this high-dose treatment and to allow the accumulation of VPL. EPI was injected 1 h after oral dosing of VPL (VPL was given at 11:00 a. m. and EPI, at 12:00 a. m.).

Blood samples (10 ml) were collected into heparinized tubes (NH_4-heparinate Monovette, Sarstedt, FRG) and centrifuged on the ward (2,000 g, 10 min), and the plasma was separated and stored in aliquots at –20° C until analysis.

Drug assay. EPI and its seven metabolites, including the two glucuronides epirubicin glucuronide (E-Glu) and epirubicinol-glucuronide (EOL-Glu), epirubicinol (EOL), and the four aglycones epirubicinol aglycon (AOLON), 7-deoxy-epirubicinol aglycon (7d-AOLON), epirubicin aglycon (AON), and 7-deoxy-epirubicin aglycon (7d-AON) [23], were measured in plasma samples using a sensitive and specific high-performance liquid chromatography (HPLC) assay developed recently [19] and slightly modified in our laboratory [24]; the lowest concentration measurable in 1-ml plasma samples was 0.1 ng/ml. VPL and nor-VPL were determined using an HPLC assay with fluorescence detection [18]; the lowest concentration measurable in 1-ml plasma samples was 5 ng/ml.

Pharmacokinetic evaluation. The concentration-time [c(t)] curves obtained for EPI were best described by a model incorporating three compartments for drug disposition, whereas those generated for VPL were best described by a model incorporating two compartments. The TopFIT software program (version 1.1) was used; this program was developed by the pharmaceutical companies Gödecke, Schering, and Thomae (FRG). The pharmacokinetic estimates were calculated using a series of differential equations, and the c(t) curves were fitted to the appropriate models by weighted, iterative, non-linear least-square regression. In addition, non-compartmental methods were used for the calculation of distribution and elimination parameters on the basis of the theory of statistical moments.

Results

Kinetic data were available for all ten subjects. The c(t) curves obtained for EPI and its glucuronide E-Glu are shown in Fig. 1. No difference in the EPI c(t) curves was found between the EPI group and the EPI+VPL group. Although the E-Glu c(t) curves were very similar, a small difference was observed that did not reach statistical significance. The c(t) curves generated for EOL and EOL-Glu show that a slight difference in EOL levels became evident after the second EPI injection, whereas a clear difference in EOL-Glu values was observed between the two treatment groups as of the the first EPI injection; this difference increased with the number of EPI injections given (Fig. 2). The c(t) curves obtained for the aglycones AON and 7d-AON (AOLON and 7d-AOLON, respectively) are depicted in Fig. 3. No difference in AOLON values was found between the two treatment groups, but a clear difference was observed for 7d-AOLON. The c(t) curves obtained in the EPI group and the EPI + VPL group were compared using the calculated AUC values in the Mann-Whitney *U*-test. Significant differences were found in only 7d-AON and 7d-AOLON levels (P = 0.048). For EOL-Glu, the P value was 0.07. P values of 0.1–0.3 were obtained for the other metabolites and for EPI.

The repetitive administration of EPI resulted in an increase in the AUC values found for the metabolites after the second and third injections of EPI that was much more pronounced in the EPI + VPL group (Figs. 4, 5). Student's *t*-test for paired data (AUC, first versus third administration) revealed significant differences (P <0.05) for E-Glu (both treatment groups), EOL-Glu (only in the VPL treat-

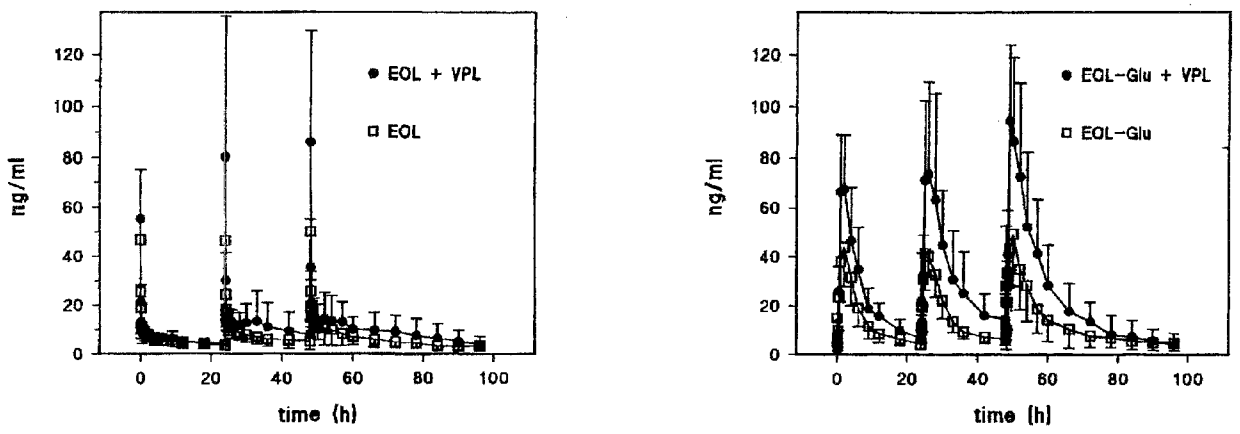

Fig. 2. C(t) curves obtained for EOL and EOL-Glu during treatment of patients with EPI alone or in combination with VPL. Data represent mean values ± SD; differences are not statistically significant ($P = 0.07$ for EOL-Glu)

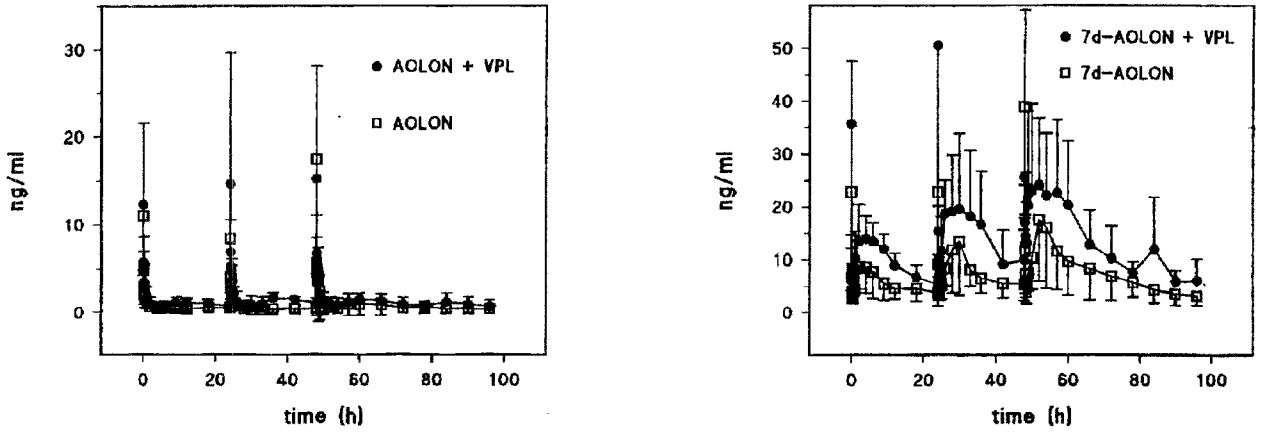

Fig. 3. C(t) curves obtained for AOLON and 7d-AOLON during treatment of patients with EPI alone or in combination with VPL. Data represent mean values ± SD; differences between AOLON values are not statistically significant ($P = 0.048$ for 7d-AOLON)

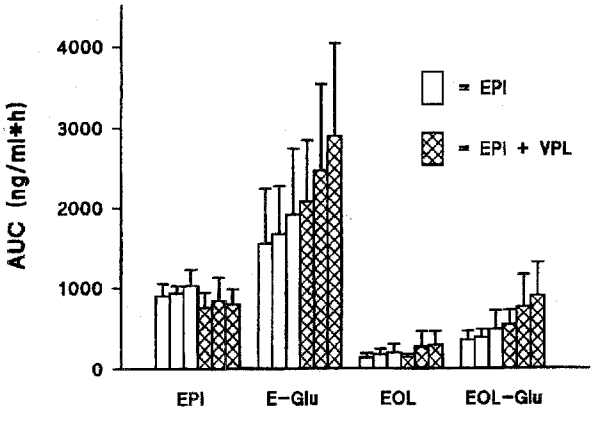

Fig. 4. AUC values obtained for EPI, E-Glu, EOL, and EOL-Glu after each of the three consecutive EPI injections (0–24 h) within one treatment cycle. Data represent mean values ± SD. *Hatched area*, Co-administration of VPL

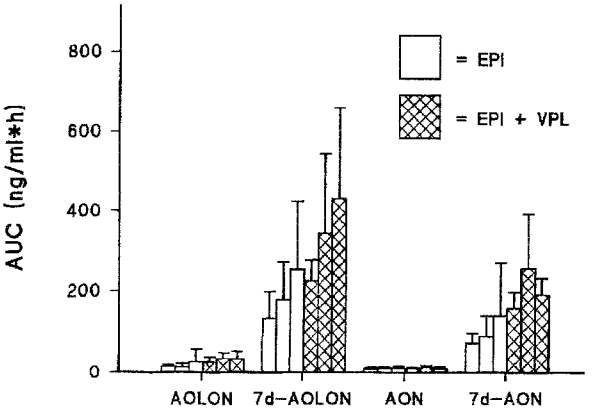

Fig. 5. AUC values obtained for AOLON, 7d-AOLON, AON, and 7d-AON after each of the three consecutive EPI injections (0–24 h) within one treatment cycle. Data represent mean values ± SD. *Hatched area*, Co-administration of VPL

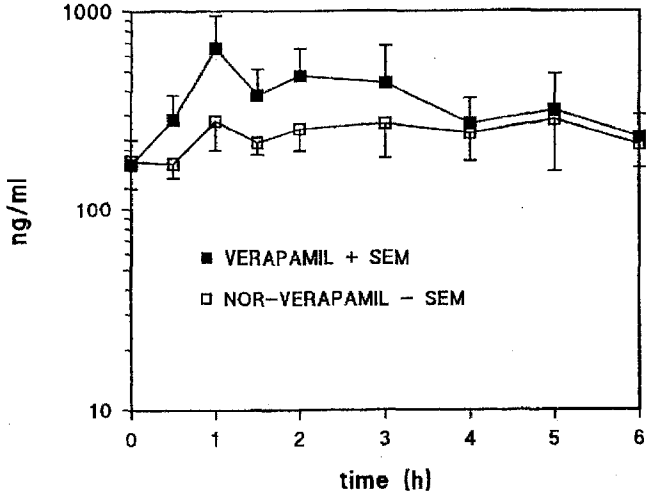

Fig. 6. C(t) curves obtained for VPL and nor-VPL after the 2nd oral administration of VPL on day 0 (see Patients and methods). Data represent mean values (+ SEM for VPL and – SEM for nor-VPL)

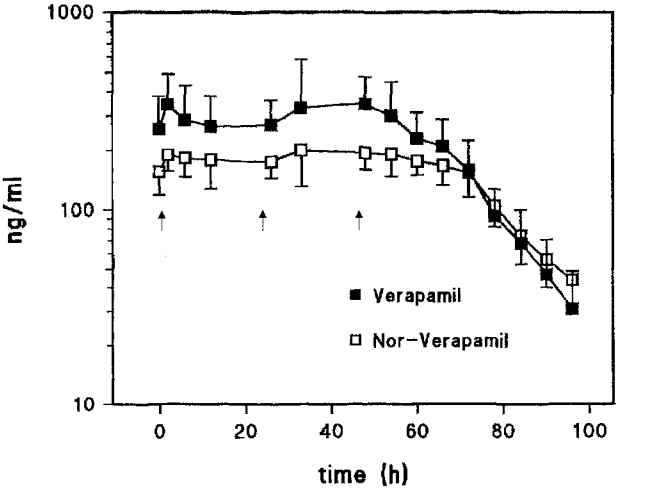

Fig. 7. C(t) curves obtained for VPL and nor-VPL during EPI treatment. *Arrow*, EPI injection. Data represent mean values (+ SEM for VPL and – SEM for nor-VPL)

Table 1. Pharmacokinetics of EPI and its metabolites in patients receiving EPI alone or in combination with VPL

	AUC (ng/ml*h)	$t_{1/2(term.)}$ (h)	MRT (h)	Vdss (l/kg)	Clp (ml/min/m²)
EPI+VPL	3400 (701)	25.5 (0.6)	21.7 (9.8)	38.6 (13.7)	1159 (542)
EPI	4080 (691)	27.0 (5.8)	22.2 (5.7)	36.4 (12.3)	993 (127)
E-Glu+VPL	7856 (3174)	25.9 (18.8)	24.6 (18.3)		
E-Glu	5570 (2283)	24.2 (22)	23.8 (23)		
EOL-Glu+VPL	2382 (1126)	18.7 (9.7)	20.9 (10.1)		
EOL-Glu	1341 (491)	28.4 (13.5)	30.6 (16.5)		
EOL+VPL	845 (538)	23.1 (5.9)	49.6 (32.6)		
EOL	586 (251)	29.5 (16.0)	34.8 (15.0)		
Aolon+VPL	115 (63)	12.9 (4.9)			
Aolon	67 (52)	13.8 (12.9)			
7d-Aolon+VPL	1208 (531)	18.5 (4.1)			
7d-Aolon	676 (362)	22.4 (12.0)			
Aon+VPL	39 (10)	1.21 (1.9)			
Aon	44 (13)	5.9 (8.2)			
7d-Aon+VPL	694 (222)	18.0 (15.0)			
7d-Aon	336 (220)	12.0 (6)			

Data represent mean values; numbers in parentheses indicate the SD

Table 2. Pharmacokinetics of VPL and its metabolite nor-VPL in patients receiving EPI and VPL

	t_{max} (h)	C_{max} (ng/ml)	AUC (ng/ml*h)	$t_{1/2lterm.}$ (h)	MRT (h)	Clp (ml/min/m²)	Vdss (l/kg)
VPL[1]	1.0 (0.4)	705 (473)	1859 (1214)	2.9 (0.7)	5.3 (0.9)	15.5 (11.1)	3.5 (1.8)
nor-VPL[1]	1.1 (0.2)	308 (122)	1294 (702)	8.0 (2.4)	12.1 (3.4)		
		C_{mean}	AUC	$t_{1/2\,term}$			
VPL	–	265 (42)[2]	19387 (6646)[2]	9.9 (2.7)[3]	–	–	–
nor-VPL	–	180 (12)[2]	14575 (2638)[2]	14.6 (3.8)[3]	–	–	–

[1] PK estimates after end VPL administration

[2] mean steady state concentration and area under the curve from start of EPI injections at day 1 to day 4 including totally 12 VPL administrations

[3] $t_{1/2}$ (elimination phase) after totally 16 VPL administrations

VPL, verapamil; nor-VPL, nor-verapamil, t_{max}, time when maximal concentration was observed; C_{max}, maximal concentration; AUC, area under the curve; $t_{1/2}$ term., half-life of the elimination phase; MRT, mean residence time; Clp, plasma clearance; Vdss, volume of distribution at steady state; C_{mean}, concentration at steady state

ment group) and 7d-AOLON (only in the VPL treatment group). The extent of metabolism was assessed using the formula $AUC_{EPI}/AUC_{EPI} + AUC_{\Sigma\ metabolites} \times 100$. A significant difference ($P < 0.05$, Student's t-test for grouped data) was found between the EPI group and the EPI + VPL group (22% ± 6% and 34% ± 7%, respectively). The kinetic data are summarized in Table 1.

In five patients we studied the pharmacokinetics of VPL after the second oral dose (therefore, VPL was present in the first sample at the time of oral intake). The c(t) curves (mean values ± SEM) are depicted in Fig. 6. The peak plasma concentration was achieved after 1 h, and the VPL levels varied between 200 and 700 ng/ml. Nor-VPL levels were always lower. The kinetic data are shown in Table 2. For the determination of c(t) curves for VPL and nor-VPL during the 4-day treatment period in the five patients receiving EPI + VPL, we used the same plasma samples that had been drawn for the EPI kinetics study. From these data we recognized an extreme variation in the $AUC_{0-96\,h}$ values for VPL and nor-VPL. Figure 7 shows these c-(t) curves (mean values ± SEM). In the group of patients studied, the mean concentration of VPL was 265 ± 42 ng/ml and the mean level of nor-VPL was 180 ± 12 ng/ml. The terminal half-lives calculated for VPL and nor-VPL after 16 doses of VPL were 9.9 ± 2.7 and 14.6 ± 3.2 h, respectively.

Discussion

The present study seems to indicate a pharmacokinetic interaction between epirubicin and verapamil. The AUC, terminal half-life, mean residence time, volume of distribution, and plasma clearance of EPI were not affected by the additional administration of VPL, whereas differences were found in its metabolism. These findings are markedly different from the results obtained by Kerr et al. [17] in similar pharmacokinetics and metabolism studies in patients receiving doxorubicin alone or in combination with VPL. These authors found that following the combination treatment, doxorubicin peak levels, the terminal half-life, and the volume of distribution at steady state were higher, whereas plasma clearance and the volume of the central compartment were lower.

No significant difference in AON or AOLON levels was found between the two treatment groups; small differences were observed in E-Glu and EOL levels, but these differences failed to reach statistical significance due to the small number of patients involved and to the marked interpatient variation, which resulted in a relatively large standard deviation. Significant differences were found in levels of the 7-deoxy-aglycones (7d-AOLON and 7d-AON), and the differences observed in EOL-Glu concentrations approached significance. Due to the small number of patients included in each group, we used a nonparametric statistical method. As mentioned above, significant differences were found in levels of the 7-deoxy-aglycones. The AUC values were always higher in the EPI + VPL group, possibly indicating an enhancement of the formation of these metabolites due to enzyme induction or to the increase in liver blood flow induced by VPL. Furthermore, a reduction in plasma clearance due to blocking of the p170-glycoprotein in the normal liver, gut, and kidney (where this protein acts as an excretion system for xenobiotics) can alter the AUC values obtained for anthracyclines. However, this explanation would not be sufficient to explain why not all anthracyclines are affected, assuming that the pump does not have different transport capacities for the various metabolites. Therefore, drug interaction with the enzyme systems necessary for metabolism of the specific compound as well as the influence of VPL on the liver blood flow may offer a better explanation for these results.

Obviously, evidence of significantly altered routes of drug metabolism emerged in the present study as judged from the comparison of the ratio $AUC_{EPI}/AUC_{EPI+AUC\Sigma_{metabolites}}$. The major contributory metabolite was E-Glu, which is generated by conjugation of the 4′-hydroxy group of EPI with glucuronic acid, followed by the 7-deoxy-aglycones, particularly 7d-AOLON, which occurred in significant amounts. The latter metabolites are formed by complex enzyme-catalyzed bioreduction [4], which has been linked to the evolution of reactive and free-radical intermediates [22]. From this viewpoint, this result may be an important indicator of pharmacological activity in humans, which has recently been related more to detoxification processes than to anticancer activity [5]. EOL, which is not the major contributory metabolite in EPI's metabolism in contrast to doxorubicinol (AOL) in the case of doxorubicin [23], is produced by ubiquitous cytoplasmic reductase enzyme systems dependent on reduced nicotinamide adenine nucleotide phosphate [10].

VPL is a vasodilator that has been shown to increase the hepatorenal blood flow in humans [20]. This effect may have contributed to the observed alterations in EPI metabolism, but it cannot thoroughly explain why not all metabolites were affected. A higher clearance of EPI was not found in patients treated with EPI combined with VPL. The contribution of the increase in hepatic blood flow to the observed pharmacokinetic results remains elusive but is considered to be a factor in the observed deviations.

The pharmacokinetics of EPI given repetitively on 3 consecutive days in the absence of VPL was very similar to what is known from i. v. bolus injections [23]. The split-dose administration of EPI has some interesting features. At three times rather than one, cancer cells are exposed to high plasma drug levels; in the present study, drug concentrations of >10 ng/ml were observed to persist for at least 96 h. Thus, the split-dose schedule mimics two opposing conditions: the achievement of high plasma concentrations for a short time and the persistence of low drug levels over a period of several days. A slight increase in the AUC values obtained for metabolites became evident in a comparison of the $AUC_{0-24\,h}$ values resulting from the three injections, indicating a small but apparent cumulative effect, possibly related to an induction of enzyme systems involved in the complex metabolism of EPI given in repetitive injections, which was statistically significant (first versus third drug injection) for E-Glu in both treatment groups and for EOL-Glu and 7d-AOLON only in the VPL-treated group. In comparison with previous reports of EPI pharmacokinetics after i. v. bolus injection [28], the terminal half-

life, volume of distribution, and plasma clearance were greater following split-dose administration, and the AUC value was 2-fold that resulting from i.v. bolus injection [31]. Assuming that the cytostatic effect is proportional to the product of extracellular drug concentration and exposure time [9], the split-course administration mode should be more effective in terms of anticancer efficacy. Such a split-course schedule for high-dose EPI (50 mg/m^2 given on 3 consecutive days) will be investigated by the European Organization for Research and Treatment and its Cancer-Soft Tissue and Bone Sarcoma Group (EORTC-STBSG) in metastatic soft-tissue sarcomas [33].

The pharmacokinetics of VPL was similar to what is known [8]. The pharmacokinetics was best described by an open two-compartment model. VPL is rapidly cleared from the system, and its elimination is dependent on the liver blood flow. The liver is the major site of biotransformation. We observed terminal half-lives for VPL and nor-VPL that were similar to those described in the literature [14]. After the 4-day treatment with VPL, the terminal half-lives of VPL and nor-VPL became longer. This finding is in line with the observation that the clearance is reduced after multiple-dose VPL administration and the bioavailability (range, 10%–30%) is increased, probably due to the saturation of first-pass metabolism [14]. The resistance-modifying power of a drug depends on the maintenance of high concentrations over a longer period. We achieved steady-state plasma concentrations of about 600 ng/ml at best and those of 300 ng/ml in the worst case (VPL + nor-VPL levels). The major plasma metabolite nor-VPL, which has negligible pharmacological activity, was as effective as the parent drug in enhancing the cytotoxicity, suggesting an additive effect for VPL and nor-VPL [13]. Such relatively low concentrations in comparison with those used in in vitro studies can induce resistance modification, as has recently been reported [2, 6, 7, 21, 26, 27], but in our randomized clinical phase II study comparing EPI ± VPL in patients with advanced breast cancer, we found no clinical evidence that drug-resistance modulation took place. The response rates as well as the overall duration of survival obtained in the two treatment groups were identical, and the same held true for the toxicity encountered [25]. Myelosuppression was not enhanced by the addition of VPL, a finding that is in line with the pharmacokinetic results obtained in the present trial and with those achieved in in vitro studies [1].

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