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The pharmacokinetics of epirubicin and docetaxel in combination in rats

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Abstract *Purpose*: The aim of the present study was to investigate possible pharmacokinetic interactions between epirubicin (EPI) and docetaxel (DTX) in rats. *Methods*: Male Sprague Dawley rats ($n = 36$) were used in the study. They received either DTX (5 mg/kg, $n = 9$), EPI (3.5 mg/kg, $n = 13$), or a combination (5 mg/kg + 3.5 mg/kg, $n = 14$), administered as intravenous bolus doses. Blood samples were collected at various time-points between 3 min and 45 h after dose administration. DTX and EPI plasma concentrations were determined by HPLC analysis. Pharmacokinetic evaluation was carried out using the NONMEM program. *Results*: A three-compartment model best described the concentration-time profiles for EPI. Clearance (CL), intercompartmental clearances (Q2 and Q3), central (V1) and peripheral (V2 and V3) volumes of distribution were estimated as 3.57 l/h per kg, 5.01 l/h per kg, 12.48 l/h per kg, 0.805 l/kg, 3.67 l/kg and 158 l/kg, respectively. A two-compartment model was sufficient to describe the DTX data. CL, intercompartmental clearance (Q), V1 and V2 for DTX were estimated as 7.3 l/h per kg, 4.6 l/h per kg, 0.69 l/kg and 2.6 l/kg, respectively. No significant change in the disposition of either drug was found when they were administered in combination compared to when they were given singly. *Conclusion*: Concurrent treatment with EPI and DTX does not appear to cause any changes in the pharmacokinetics of the drugs in rats.

Key words Docetaxel · Epirubicin · Interaction · Pharmacokinetics · Rats

Introduction

Epirubicin (EPI) is an anthracycline and a stereoisomer of doxorubicin. It is highly effective against various types of cancer, both when used as a single drug and as part of combination therapies [8, 19]. The pharmacokinetics and pharmacodynamics of EPI have been studied extensively over the years [6, 21, 22]. The major side effects of EPI are haematological and cardiac toxicity. It is believed that the risk of cardiotoxicity increases with increasing accumulated dose [19], whereas grade of haematological toxicity seems to be related to a single exposure to the drug [12]. Hence, since EPI most often is given in combination therapies, possible pharmacokinetic interactions are important to study. EPI combinations studied from this point of view are, among others, EPI-paclitaxel [5], EPI-quinine [7] and fluorouracil-EPI-cyclophosphamide [24].

During the last decade the taxanes, a group of drugs with promising chemotherapeutic activity have become members of the anticancer arsenal of drugs. Paclitaxel and docetaxel (DTX) have both shown activity against various types of tumours. Although the taxanes have been administered mostly as single drugs, they have also during the last few years been given as part of combination therapies [1, 8].

Since both EPI and DTX are among the most active drugs in the treatment of breast cancer, it is not surprising that they have been evaluated as combination therapy in that setting [14]. Although pharmacokinetic interactions between anthracyclines and taxanes have been reported [1, 5], there is to our knowledge no study on the EPI-DTX combination. The aim of the present study was to study possible pharmacokinetic interactions between EPI and DTX in rats.

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Materials and methods

Animals

A total of 36 male Sprague Dawley rats (Charles River, Upsala, Sweden) were used in the study. After 1 week of acclimatization, catheters were inserted into the arteria carotis and vena jugularis. Inhalation of enflurane, supplied as Efrane (Abbott Scandinavia AB, Sweden), mixed with 1.5% nitrous oxide and 1.5% oxygen, was used for anaesthesia during the surgery. The pharmacokinetic

experiments were carried out 2–3 days after surgery. The body weights of the rats were then on average 269 g (range 228–310 g). The rats had free access to standard pellet food and water, even during the experiments. At the end of the experiment the rats were killed using CO_2. The Animal Ethics Committee in Upsala approved the study.

Drug administration

EPI, supplied as Farmorubicin 10 mg dry substance for injection (Pharmacia & Upjohn, Sweden), was diluted with sterile water (Pharmacia & Upjohn, Sweden) to a concentration of 2 mg/ml. DTX was supplied as Taxotere 20 mg (Rhône-Poulenc Rorer, France). The infusion concentrate (20 mg DTX in 0.5 ml polysorbate 80) was diluted in 1.5 ml 13% ethanol in sterile water, that was enclosed with Taxotere infusion concentrate to a concentration of 10 mg/ml. This solution was further diluted with sodium chloride 9 mg/ml (Pharmacia & Upjohn, Sweden) to a final concentration of 2.5 mg/ml.

The rats were randomly divided into three groups (Table 1). Group 1 ($n = 13$) received EPI 3.5 mg/kg alone, group 2 ($n = 9$) received DTX 5 mg/kg alone, and group 3 ($n = 14$) received the drugs in combination at the same doses as groups 1 and 2. The selected doses have previously been given to rats with tolerable toxicity [4, 16]. The drugs were given as intravenous bolus injections into the vena jugularis catheter. In order to mimic the clinical situation, EPI was given first, immediately followed by DTX. When only one of the drugs was administered, the corresponding volume of sodium chloride 9 mg/ml (Pharmacia and Upjohn, Sweden) was given in place of the other drug.

Blood sampling

Blood samples of 250–1000 μl for measurement of EPI and DTX levels were withdrawn from the arterial catheter at various times between 3 min and 45 h after the bolus injections (Table 1). The sampling times were selected so as to cover the main parts of the concentration-time profiles of both drugs. If the artery catheter was not functioning, the blood samples were instead taken from the hind paw; 48 samples out of a total of 160 samples were drawn in this way. The samples were collected in EDTA Microtainer tubes (Becton Dickinson, Sweden) and put on ice for a couple of minutes before being centrifuged for 3 min at 10,000 rpm in a prechilled centrifuge. The plasma was collected in 1.5 ml polypropylene tubes (Treff AG LABORA, Sweden) and stored at −80° C until analysis. After sampling, the catheter was rinsed with 0.2–0.3 ml sodium chloride, 9 mg/ml (Pharmacia and Upjohn, Sweden).

Table 1 Administered doses and number of blood samples

	Group 1 ($n = 13$)	Group 2 ($n = 9$)	Group 3 ($n = 14$)
Number of samples per individual			
Average	5.2	4.2	3.9
Range	3–8	3–6	3–7
Epirubicin			
Dose (mg/kg)			
Average (± SD)	3.55 ± 0.27		3.6 ± 0.31
Range	3.15–3.98		3.21–4.33
Number of samples available for drug measurement			
Total	65		54
Hind paw	19		14
Docetaxel			
Dose (mg/kg)			
Average (± SD)		5.05 ± 0.25	5.13 ± 0.39
Range		4.60–5.47	4.69–5.97
Number of samples available for drug measurement			
Total		38	27
Hind paw		15	3

Chemical analysis

Plasma samples of 150 μl were used in the analyses. When less than 150 μl plasma was obtained, the samples were diluted with drug-free rat plasma. EPI was determined as previously described [11, 15, 20], with minor modifications [24]. The between- and within-run coefficients of variation, measured at four concentrations (20–600 ng/ml), were less than 7%, but at 5 ng/ml was 13%. The accuracy was 86–103% at concentrations in the range 20–600 ng/ml and 94% at 5 ng/ml. DTX plasma levels were analysed according to a previously described method, originally developed for determination of paclitaxel in human plasma [26]. The between- and within-run coefficients of variation, measured at three concentrations (300–7800 ng/ml), were less than 14%, but at 170 ng/ml was 20%. The accuracy was 94–101% at concentrations in the range 200–7800 ng/ml and 104% at 170 ng/ml.

Pharmacokinetic analysis

The pharmacokinetic models best describing the concentration-time profiles of DTX and EPI were determined by population analysis using the mixed effects models in the program NONMEM version V [3]. Lognormal residual error models were used according to the following equation:

$$\mathrm{COBS} = \mathrm{CPRED} \times e^{\varepsilon} \quad (1)$$

where COBS and CPRED denote the observed concentration and model-predicted concentration and residual error, respectively, and ε is a symmetrically distributed, zero-mean variable with variance sigma, which is estimated as part of the model.

Combination treatment was introduced stepwise as a covariate in the models and evaluated as a significant influence on the pharmacokinetic parameters of both drugs. The effect of the covariates was tested on one parameter at a time and in addition on all the parameters of each model simultaneously. The model building process was guided by graphical evaluation within the program Xpose, version 2.2 [13], as well as by the objective function, produced by NONMEM, which is a goodness of fit statistic. For hierarchical models a drop in the objective value of more than 3.84 denotes an improved fit at the $P < 0.05$ level for a one-parameter difference. NONMEM also produces individual parameter estimates, which are obtained as empirical Bayes estimates using the final model. Individual predicted concentrations are based on those parameter estimates.

Results

EPI

A total of 119 EPI measurements from 27 rats were available for pharmacokinetic population analysis (Table 1). The observed concentrations versus time are shown in Fig. 1A. The concentration-time profile for one of the rats in group 3 appeared unlikely since it showed initially low but increasing concentration with time. Erroneous administration was concluded and data from that rat were therefore excluded from further analysis.

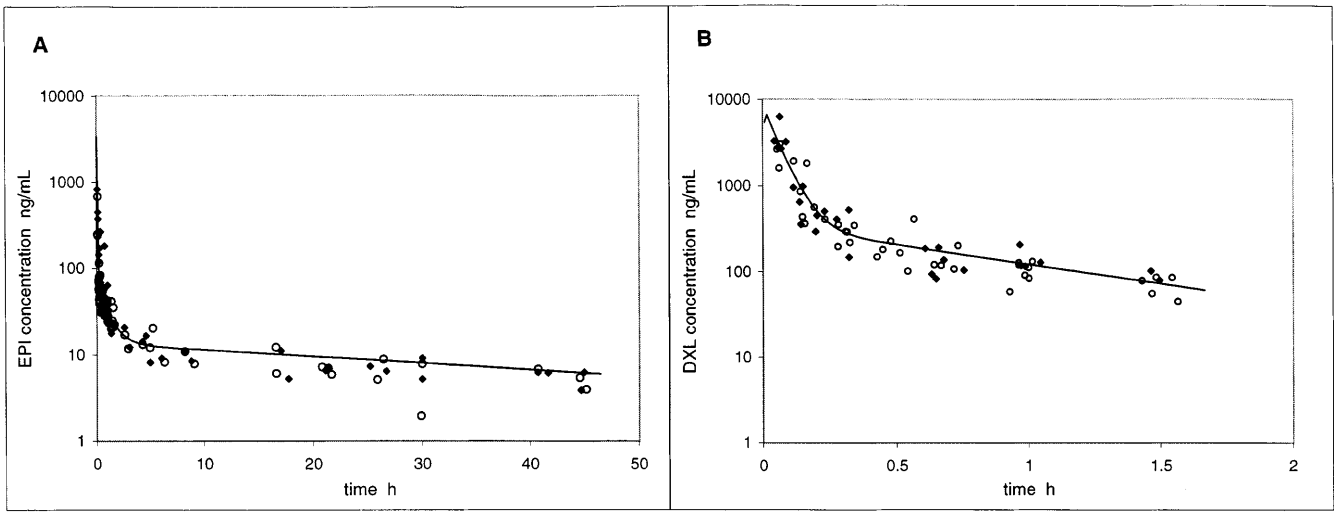

Fig. 1A,B Observed concentrations versus time for EPI (**A**) and DXL (**B**) in rats that received single-drug treatment (*empty circles*) and combination-drug treatment (*filled diamonds*). The *solid lines* represent the model-predicted concentration-time profiles in the typical individuals

The optimal structural model describing the data was a three-compartment model. In order to detect any possible difference between the two sites of sample collection, hind paw sampling was initially introduced as a covariate in the model. The EPI pharmacokinetic parameters estimated from the samples taken from the hind paw were not significantly different from the others with one exception. One of the peripheral volumes of distribution (V3) from the hind paw samples was estimated as only 70% of the corresponding estimate from the arterial samples. None of the parameters in the model were found to be significantly affected by co-treatment with DTX.

The population parameters obtained in the final model are shown in Table 2. The initial, intermediate and terminal half-lives, which are secondary parameters in the model, were estimated as 0.03, 0.70 and 40.0 h, respectively. The EPI concentrations, predicted by the final model, were in good agreement with those observed (Fig. 2A,B). The model-predicted concentration-time profile in a typical individual is shown in Fig. 1A.

DTX

A total of 65 DTX determinations from 19 rats were available in the DTX pharmacokinetic analysis (Table 1). The observed concentration-time data are displayed in Fig. 1B. The profile for one of the rats in group 3 (not the same rat as the one with an unrealistic EPI concentration-time profile) was extreme in comparison with the others with a very high initial concentration and apparently short initial half-life. The data from that rat were therefore excluded from the pharmacokinetic analysis.

A two-compartment model was found to best describe the DTX data. No significant improvement was obtained when hind paw sampling was introduced into the model. Furthermore, none of the parameters in the basic model was significantly influenced by coadministration of EPI.

The pharmacokinetic parameters in the final model are shown in Table 2. Initial and terminal half-lives were estimated from those parameters as 0.04 and

Table 2 Population parameters in the final models (CL total clearance; *V1*, *V2*, *V3* central and peripheral volumes of distribution, respectively; *Q*, *Q2*, *Q3* intercompartmental clearances, *Rel SE %* standard error as percentage of parameter value)

Drug	Parameter	Estimate		Interindividual variability	
		Value	Rel SE %	%	Rel SE %
Epirubicin	CL (l/h/kg)	3.57	12	42	49
	Q2 (l/h/kg)	5.01	38		
	Q3 (l/h/kg)	12.48	19		
	V1 (l/kg)	0.805	28	25	48
	V2 (l/kg)	3.67	41		
	V3 (l/kg)	158	14		
	Residual variability (%)	16	9		
Docetaxel	CL (l/h/kg)	7.32	7	18	51
	Q (l/h/kg)	4.61	16		
	V1 (l/kg)	0.694	16		
	V2 (l/kg)	2.59	17		
	Residual variability (%)	28	8		

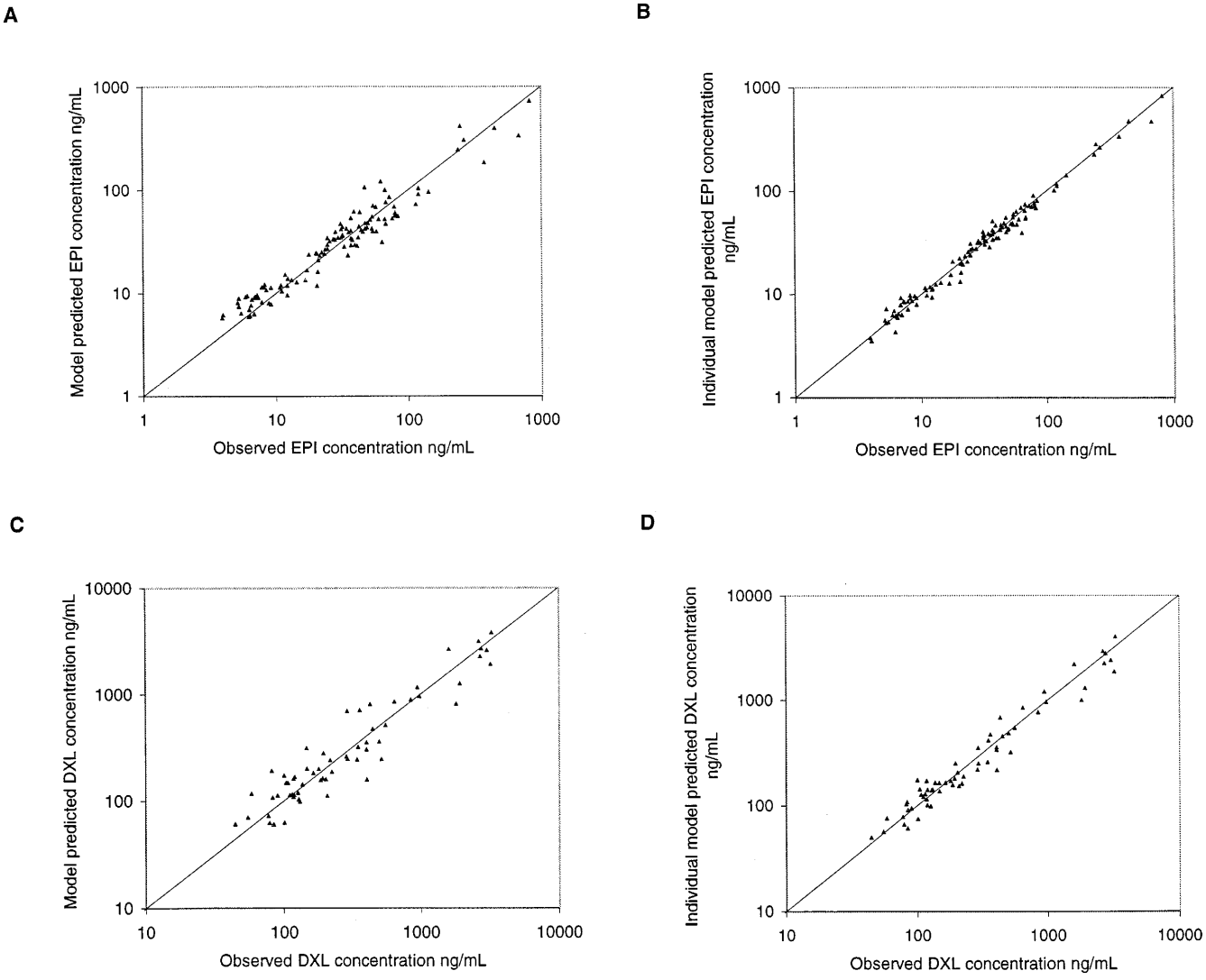

Fig. 2A–D Model-predicted versus observed concentrations for EPI (**A, B**) and DXL (**C, D**). Solid lines are lines of identity

0.7 h, respectively. The extreme individual was not included in the final model. However, when that individual was included, the pharmacokinetic parameter estimates were unaffected, but the standard errors of the estimates were approximately doubled. Predicted concentration-time profile for the typical individual by the final model is displayed in Fig. 1B. Model-predicted versus observed DTX concentrations are displayed in Fig. 2C,D.

Discussion

The large variability in pharmacokinetics between and within individuals is one of the reasons for weak dose-response relationships in anticancer therapy; the variability in concentration-response relationships is another. Since most anticancer agents are administered as combination therapies, possible pharmacokinetic interactions will make the relationships even more obscure and therefore these are important to evaluate.

In both humans and rats, DTX is mainly metabolized by the cytochrome P-450 isoenzymes of the CYP3 A subfamily [4, 17]. It is known that epirubicin in humans primarily either undergoes conjugation followed by hepatobiliary elimination or is converted to epirubicinol by an aldoketoreductase [21]. However, it has not yet been clarified whether a CYP3 A enzyme is involved in the elimination process.

It has been shown that cyclosporine, which is a substrate for CYP3A4, decreases the CL of doxorubicin in humans by 40–50% [2, 23]. A possible explanation for this could be that CYP3A4 is involved in the elimination of anthracyclines and another that cyclosporine, in addition an inhibitor of P-glycoprotein (Pgp) which is involved in the biliary excretion of drugs, inhibits the biliary elimination of doxorubicin. Furthermore, DTX is a substrate for Pgp [10], and consequently a decreased EPI CL, when given in combination with DTX, is conceivable.

Since PgP is present in many tissues in humans, mice and rats and since anthracycline efflux out of cells is at least partly governed by Pgp, combinations of anthracyclines and taxanes could theoretically result in a changed distribution of the anthracycline in these species. This has been shown for EPI when combined with paclitaxel in mice [5]. In that study EPI concentrations in the heart were doubled in mice that received the combination compared to those that were given single-drug treatment. Paclitaxel was, however, dissolved and administered in Cremophor EL, yet another substrate for PgP [27] and this solvent was found to be partly responsible for the distribution changes.

This is the first study of the pharmacokinetic interactions between EPI and DTX. As stated above, there are a number of mechanisms by which pharmacokinetic interactions can take place when the two drugs are combined, in both humans and rats. There was, however, no indication of interactions between EPI and DTX in the present study.

Reports on EPI pharmacokinetics in rats are sparse in the literature. In one study EPI CL was estimated as 0.11 l/h, whereas the initial and terminal half-lives were estimated as 0.22 and 22.2 h, respectively [18]. These values should be compared to the estimate of 0.96 l/h for CL, which corresponds to 3.57 l/h per kg when corrected for the average body weight of 269 g, and half-lives of 0.03, 0.7 and 40 h obtained in the present study. The main reason for the nearly tenfold difference between the CL estimates is probably that determination of EPI in the former study was made using an unselective method, which takes total fluorescence, and thereby also the metabolites, into account.

DTX CL, volume of distribution at steady state (V1 + V2) and the initial and terminal half-lives in rats have previously been reported to be 5.5 l/h per kg, 4.0 l/kg, 0.02 h and 0.78 h [4]. Those values are in accordance with the corresponding estimates from the present study: 7.32 l/h per kg, 3.3 l/kg, 0.04 and 0.7 h, respectively.

The lack of interaction between EPI and DTX found in the present study is in agreement with the results obtained in the study on interactions between EPI and paclitaxel in mice [5]. In that study the change in distribution of EPI could not be detected in plasma, whereas when the tissues were examined separately essential differences in EPI levels between the two groups of mice (single group and combination group) were established. In the present study, the peripheral levels were not investigated, making it impossible to draw any conclusions about the absolute distribution of EPI and DTX in the tissues. Since DTX structurally resembles paclitaxel [25], it is possible that it has a similar impact on EPI distribution. However, paclitaxel is dissolved in Cremophor EL and DTX is dissolved in polysorbate 80 and since both solvents have been shown to bind to Pgp [9, 27], it is not quite clear what would be expected from the EPI-DTX combination.

In summary, this is the first investigation of the pharmacokinetic interactions between EPI and DTX. The data did not show any evidence of pharmacokinetic interaction between the two drugs.

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