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ORIGINAL ARTICLE

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Population pharmacokinetic model for daunorubicin and daunorubicinol coadministered with zosuquidar.3HCl (LY335979)

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Abstract *Purpose*: The impact of zosuquidar.3HCl, an inhibitor of P-glycoprotein, on the pharmacokinetics of daunorubicin and daunorubicinol was examined in a phase I trial using a population approach. Pharmacokinetic and pharmacodynamic properties of zosuquidar.3HCl were also determined. *Methods*: The pharmacokinetics of daunorubicin and daunorubicinol were studied following daunorubicin administration on day 1 (50 mg/m^2 i.v. infusion over 10 min) alone and on day 3 concomitantly with zosuquidar.3HCl (i.v. 200 or 300 mg/m^2 over 6 h or 400 mg over 3 h). Of a total of 18 patients entered, 16 with acute leukemia completed the study. *Results*: A three-compartment pharmacokinetic model adequately described daunorubicin concentration-time profiles. Five- and four-compartment models adequately described the daunorubicin-daunorubicinol pharmacokinetics in the absence and presence of zosuquidar.3HCl, respectively. The impact of zosuquidar.3HCl on coadministered daunorubicin was minimal, with a 10% reduction in daunorubicin clearance. The model predicted a 50% decrease in daunorubicinol apparent clearance in the presence of zosuquidar.3HCl. A direct concentration-effect relationship between zosuquidar.3HCl concentrations and inhibition of rhodamine 123 (Rh123) efflux in CD56 lymphocytes was defined by a sigmoid E_{max} model. The IC_{50} was 31.7 μg/l. The zosuquidar.3HCl dosing regimen led to concentrations in excess of the IC_{90} (169.6 μg/l) and provided maximal P-glycoprotein inhibition during the distribution phases of daunorubicin. *Conclusions*: The decrease in daunorubicin and daunorubicinol clearance in the presence of zosuquidar.3HCl likely reflects inhibition of P-glycoprotein in the bile canaliculi impeding their biliary excretion. The results need to be interpreted carefully due to the sequential nature of daunorubicin administration and analysis.

Keywords LY335979 · Daunorubicin · Daunorubicinol · Pharmacokinetics · NONMEM

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Introduction

Multidrug resistance (MDR), the ability of cancer cells to be resistant or to become resistant to chemotherapeutic agents that are structurally and functionally unrelated, may be caused by a variety of factors [2, 3, 14, 19, 21, 29]. Efforts at overcoming MDR have primarily focused on trying to inhibit P-glycoprotein (P-gp).

Clinical trials with first- and second-generation MDR modulators have been disappointing due to issues with potency, tolerability, pharmacokinetic interactions with coadministered oncolytics and efficacy [1, 6, 12, 17, 23, 27, 33, 35]. Third-generation molecules (e.g. zosuquidar.3HCl) are noncytotoxic, bind with high affinity to P-gp (K_i about 20–100 n *M*) [21], and demonstrate potent in vitro reversal activity against MDR human tumour cell lines. In addition, in vitro experiments have shown that zosuquidar.3HCl inhibits P-gp with a K_i of 59 n *M*, does not inhibit either MRP1 or MRP2 (multidrug resistance-associated proteins) and has no affinity for the liver enzymes CYP3A, CYP1A, CYP2C9, CYP2D6 at nanomolar levels [7, 8, 9, 30, 40].

In clinical studies with previous modulators, the argument has been advanced that the critical factor is

maintenance of the area under the plasma concentration-time curve (AUC) of the oncolytic [32]. In this case, the lowered dose of chemotherapy and hence lower maximum plasma concentrations (C_{max}) in combination with these modulators is offset by the effect on clearance resulting in a similar AUC. Recent evidence suggests that, for anthracyclines, it is the chemotherapeutic drug's peak concentration which is the important correlate with efficacy [38]. In contrast, the toxicity for anthracyclines is most likely a function of both peak and exposure. Taken together in order to balance the efficacy and toxicity, a shorter duration of P-gp inhibition and a full-dose chemotherapy would be desired [10].

In order to achieve the above aim, a potent specific P-gp inhibitor with an extensive and rapid distribution pharmacokinetic profile (short distribution half-life) providing a direct concentration/effect (pharmacokinetic/pharmacodynamic) relationship would be desirable. In this study, the effect of zosuquidar.3HCl on P-gp function was determined using natural killer ($CD56^+$) cells collected from the patients at predetermined times in an ex vivo assay.

The results of a previous phase I study [5] have shown that (1) increasing concentrations of zosuquidar.3HCl in excess of 200 μg/l result in maximum inhibition of P-gp function, and (2) the length of zosuquidar.3HCl infusion is related to the observed pharmacokinetic interaction with coadministered doxorubicin (DOX) and doxorubicinol (DOXOL). These learning points underpinned the design of this phase I study of intravenous (6 h or shorter infusion schedule) zosuquidar.3HCl in combination with daunorubicin (DAUN) and cytarabine in patients with acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS). The pharmacokinetic/pharmacodynamic data collected for zosuquidar.3HCl in this study were fitted using a population approach in order to determine the pharmacokinetic/pharmacodynamic relationship between plasma levels of zosuquidar.3HCl and P-gp inhibition as measured ex vivo. This was followed by the primary objective of the study; the assessment DAUN and daunorubicinol (DAUNOL) pharmacokinetics in the presence of a 6-h or shorter infusion schedule of zosuquidar.3HCl.

Methods

Patient selection

Patients with a morphologically confirmed diagnosis of AML, MDS or any hematologic malignancy (except newly diagnosed acute promyelocytic leukemia) for which the investigator deemed this chemotherapy regimen appropriate were entered into the study. This trial was approved by the relevant ethics committee at the participating medical institutions and sponsored by Eli Lilly. All participants gave written informed consent and the study was conducted in accordance with the ethical principles of the most recent version of the Declaration of Helsinki. Patients were at least 18 years of age, and met other eligibility requirements which included: (1) a resting blood pool heart scan with an ejection fraction greater than 45% (patient suffering from unstable angina, uncontrolled atrial or ventricular arrhythmias or uncompensated congestive heart failure were not enrolled in the study); and (2) a performance status of 0 to 2 on the Eastern Cooperative Oncology Group Scale (unless the patient's performance status was judged to be a direct consequence of AML or MDS). Patients did not receive any investigational agent within 4 weeks prior to enrolment into the study (with the exception of 6 weeks for hydroxyurea) and if such therapy had been given before this period, patients had to have recovered from all toxic effects. Patients had not previously been enrolled in clinical trials testing other P-gp inhibitors and had not experience any prior cytarabine-related neurotoxicity. Adequate organ function (bone marrow, liver and kidney) was required.

A total of 18 patients entered the study. One of them died before receiving study drug, and another became too ill to participate in the study. Therefore, only 16 patients completed the study.

Study design, treatment and sampling scheme

Pharmacokinetic and pharmacodynamic data were collected following the administration of DAUN (day 1 and day 3) and zosuquidar.3HCl (day 3) according to the sampling schedule presented in Table 1 and dosing scheme presented in Fig. 1. All patients received premedication with antiemetics prior to receiving DAUN according to routine clinical practice. DAUN was administered at a dose of 50 mg/m^2 by slow i.v. push on day 1, day 3 and day 5 (for a total of three doses). Zosuquidar.3HCl was initially administered

Table 1 Sampling scheme (sampling times in hours)

With a 3-h infusion of zosuquidar.3HCl		With a 6-h infusion of zosuquidar.3HCl	
Daunorubicin and daunorubicinol	Zosuquidar.3HCl	Daunorubicin and daunorubicinol	Zosuquidar.3HCl
	0 (predose)[a,b]		0 (predose)[a,b]
0 (predose)[b]	0.5[a]	0 (predose)[b]	1[a]
0.17		0.17	
0.5	1	0.5	
1.5	2	1	2
2.5	3[a]	3	4
3	3.5	4	5
3.5	4	5	6[a]
5.5	6[a]	7	8[a]
7.5	8	11	12
11.5	12[a]	14	15
23.5	24[a]	23	24[a]
47		47	48

[a]Zosuquidar.3HCl pharmacodynamic assessment
[b]Dose administration of either daunorubicin or zosuquidar.3HCl

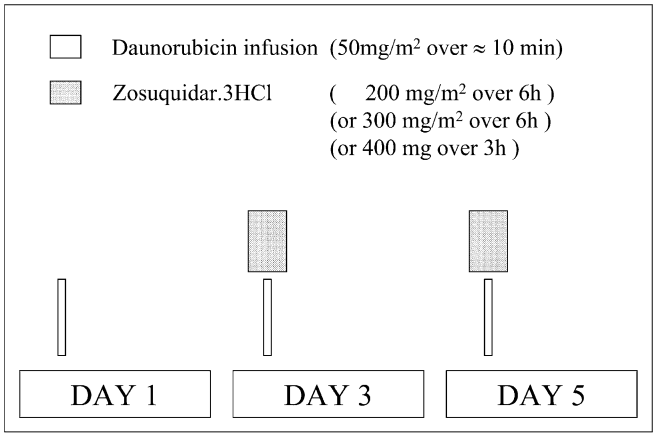

Fig. 1 Schematic representation of study design

at a starting dose of 200 or 300 mg/m^2 (6-h i.v. infusion) on day 3 and day 5 (1 h before the administration of DAUN). Previous pharmacokinetic data from another phase I [5] study had shown that dosing zosuquidar.3HCl per body surface area adversely contributes to the variability in pharmacokinetic response. Consequently, the protocol was amended to implement a flat dosing strategy. In the last cohort, patients received 400 mg (flat dosing) of zosuquidar.3HCl as a 3-h infusion on day 3 and day 5 (0.5 h before the administration of DAUN).

Biological assays

Human plasma samples were analysed for zosuquidar.3HCl using a validated LC/MS/MS method over the concentration range 10 to 500 ng/ml. The samples were analysed at Advion BioSciences, located in Ithaca, N.Y. [11].

Human plasma samples were analysed for DAUN and DAUNOL using a validated (5 to 200 ng/ml, with a limit of quantitation, LOQ, of 5 ng/ml) HPLC method using fluorescence detection [13]. The samples were analysed at PPD Development located in Richmond, Va. Samples above the LOQ were diluted and reanalysed to yield results within the calibrated range. Based on the quality control samples, the overall relative standard deviations (expression of the precision) were less than 11% and 14% for zosuquidar.3HCl and DAUN-DAUNOL, respectively. The overall relative errors (expression of the accuracy) were less than 18% and 14% for zosuquidar.3HCl and DAUN-DAUNOL, respectively.

A surrogate assay of P-gp function in patients was employed that has been previously described using peripheral blood natural killer lymphocytes that express P-gp [28]. The results are expressed as percentage inhibition of Rh123 P-gp-mediated efflux.

Data analysis

All analysis was performed with NONMEM (version V, level 1.1) using the first-order conditional linearization method with interaction [4, 20, 31, 36, 41].

Model development for Zosuquidar.3HCl PK/PD data consisted (a) of evaluating a two and three compartment PK model following zero order input kinetics, (b) exploring a direct reversible effect-concentration relationships (E_{max} and sigmoidal E_{max}) between the % inhibition of Rh123 P-gp-mediated efflux from CD56 cells and zosuquidar.3HCl concentrations.

Model development for DAUN and DAUNOL was carried out in the following sequential manner: (1) DAUN pharmacokinetic data in the absence of zosuquidar.3HCl (day 1, day 2) were modelled, and (2) using DAUN-DAUNOL data collected on day 1 and day 2 (absence of zosuquidar.3HCl), a combined parent-metabolite model was developed with DAUN pharmacokinetic parameters (population means and variances) fixed to the values of the best fit DAUN pharmacokinetic model.

The structural model tested for DAUN was a three-compartment pharmacokinetic model (previously reported to adequately describe DAUN pharmacokinetics [18]). Due to the wide range of DAUN concentrations, the natural logarithm of the concentrations was modelled. DAUNOL pharmacokinetics were modelled first using a four-compartment model (three compartments for the parent compound) with the metabolite compartment (fourth compartment) being linked to the parent central compartment and further model development was carried out depending on the data.

The pharmacokinetic data following the second administration of DAUN on day 3 (coadministered with zosuquidar.3HCl) were added to the data set. The impact of zosuquidar.3HCl on DAUN clearance (CL) and DAUNOL apparent clearance (CLm/fm, fm being the fraction of DAUN dose converted into DAUNOL) was modelled as a categorical covariate (presence versus absence of zosuquidar.3HCl). Due to the small number of patients, no other covariate relationship was explored. The modelling of DAUN-DAUNOL pharmacokinetic data on day 1 and day 3 was carried out under the principle of superposition (making the assumption that the pharmacokinetics of both parent and metabolite are linear over time and concentration).

For all pharmacokinetic models, the structure for the random effects was as follows: (a) the departure of individual pharmacokinetic parameter estimates from the corresponding population mean estimate (interindividual and interoccasion variability) was modelled according to an exponential relationship; and (b) the departure of the model predictions from the observations (residual variability) was modelled according to a proportional relationship and also in part reflects the bioanalytical error.

For the zosuquidar.3HCl pharmacokinetic/pharmacodynamic model, interindividual variability on IC_{50}(zosuquidar.3HCl concentration resulting in 50% of the maximal inhibition of Rh123 P-gp-mediated efflux) was modelled according to an exponential relationship and residual variability was modelled as an additive error.

Model selection was based on a number of criteria, such as the exploratory analysis of the goodness of fit plots, the estimates and the confidence intervals of the fixed and random parameters, and the value of the objective function. The relationship between zosuquidar.3HCl and DAUN-DAUNOL pharmacokinetics was tested using NONMEM for statistical analysis according to the criteria described by Troconiz et al. [37] (the difference in the minimum value of the objective function between a model with and without a specific covariate relationship was compared with a χ^2 distribution in which a difference greater than or equal to 7.88 points was significant at $P < 0.005$ (for one degree of freedom). Finally, based on mean and variance parameters from the final model, 1000 Monte-Carlo simulations were carried out in order to generate the 95% population prediction interval.

Results

Zosuquidar.3HCl pharmacokinetics

Two- and three-compartment pharmacokinetic models were tested to describe zosuquidar.3HCl pharmacokinetics. The goodness of fit plots (Fig. 2) showed that a two-compartment pharmacokinetic model adequately fitted the data. The three-compartment pharmacokinetic model gave a similar result (data not shown). However, given that, (a) the majority of individual profiles displayed biexponential disposition kinetics and, (b) the parameters for the three-compartment model were not as precisely estimated compared to the two compart-

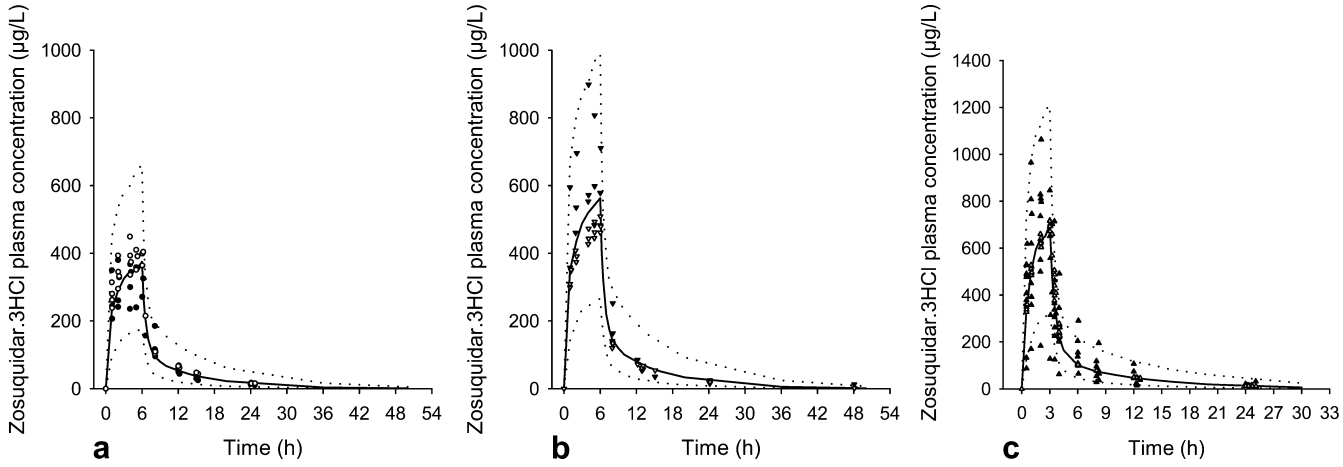

Fig. 2a–c Zosuquidar.3HCl observed (*closed symbols*) and predicted (*open symbols*) plasma concentration versus time profiles following (**a, b**) a 6-h i.v. infusion of (**a**) 200 mg/m^2 or (**b**) 300 mg/m^2, or (**c**) a 3-h i.v. infusion of 400 mg/m^2. The *solid* and *dotted lines* represent the median pharmacokinetic profiles and the 95% population prediction interval calculated from 1000 Monte-Carlo simulations

mental PK model, the latter model was retained as the final model. The corresponding pharmacokinetic parameters are presented in Table 2.

Zosuquidar.3HCl pharmacodynamic model

Zosuquidar.3HCl pharmacodynamic data were best fitted by a sigmoidal E_{max} relationship with a Hill coefficient of 1.31 and the E_{max} fixed to 100% (Table 2). From this model, the $IC_{50}$was 31.7 µg/l and the IC_{90} was 169.6 µg/l (mean). The same model when the E_{max} was estimated (not fixed) gave very similar results. A zosuquidar.3HCl plasma concentration in excess of the IC_{90} was readily achieved with both the 3-h and the 6-h infusions. Figure 3 displaying the pharmacodynamic versus time profiles shows that maximal P-gp inhibition (>90%) was maintained for approximately 7 h and 4 h for the 6-h and 3-h infusions, respectively. The parameters and goodness of the fit plots for this model are presented in Table 2 and Fig. 3, respectively.

Daunorubicin pharmacokinetics

The DAUN data were adequately described by a three-compartment pharmacokinetic model. The pharmacokinetic parameters and corresponding goodness of fit plots are presented in Table 3 and Fig. 4, respectively. The goodness of fit plots showed no evidence of model misspecification. Table 3 shows an average 10% reduction in DAUN CL in the presence of zosuquidar.3HCl. In addition, a model estimating independently the percentage decrease in DAUN CL for the 3-h and 6-h infusions, 8% (precision 52%) and 15% (precision 25%), respectively, gave a very similar fit and objective function compared to the final model, and therefore was not used further. The impact of zosuquidar.3HCl on DAUN peripheral volume of distribution (V2) was tested and was not found to significantly improve the model.

Daunorubicinol pharmacokinetics

Figure 5 shows the typical DAUNOL pharmacokinetic profiles in the presence and absence of zosuquidar.3HCl with a double peak in the absence of zosuquidar.3HCl, which was not observed in the presence of zosuquidar.3HCl (Table 4). As expected from previous work on anthracyclines [5], these profiles

Table 2 Zosuquidar.3HCl pharmacokinetic parameters from the two-compartment population pharmacokinetic model and pharmacodynamic parameters from the sigmoidal E_{max} model (*SEE* standard error on the estimate)

Parameter	Population mean estimate (%SEE)	Interpatient variability estimate (%) (SEE%)
PK parameters		
Clearance, CL (l/h)	127 (9.45)	35.1 (56.0)
Central volume of distribution, V1 (l)	127 (16.5)	62.0 (51.4)
Intercompartmental clearance (l/h)	79.9 (11.9)	Not estimated
Peripheral volume of distribution, V2 (l)	412 (7.0)	11.3 (49.4)
Covariance CL-V1 (%)		43.5 (62.4)
Proportional error (SE %)	27.0% (9.3)	
PD parameters[a]		
E_{max}(%)	100 fixed	
LY_{50}(µg/l)	31.7 (32.2)	103 (30.0)
γ (Hill coefficient)	1.31 (11.7)	
Additive residual error (SEE%)	13.5% (57.9)	

[a]PD parameters relate to the following equation (LY zosuquidar.3HCl concentration): % inhibition Rh123 efflux = E_{max} *$(LY)^{\gamma}/((LY_{50})^{\gamma}+(LY)^{\gamma})$

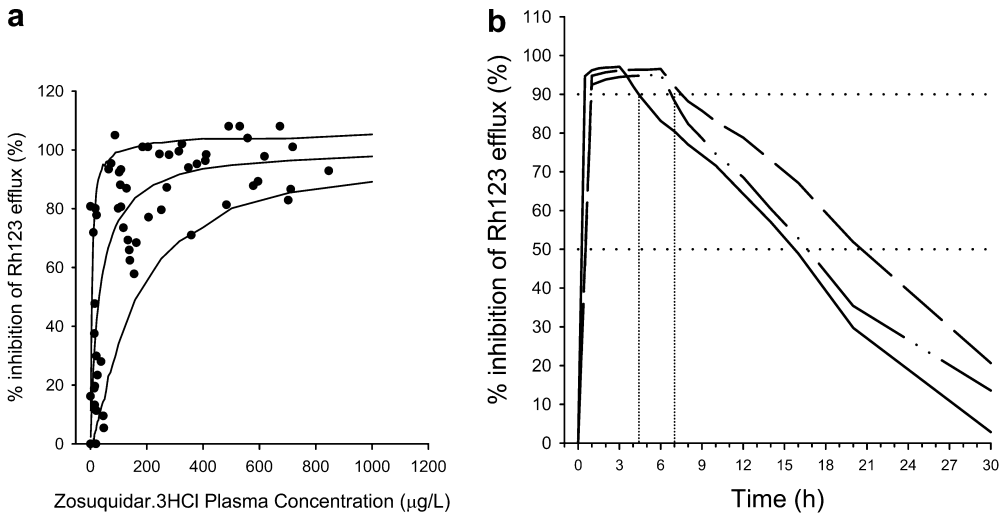

Fig. 3 **a** Percentage inhibition of Rh123 P-gp-mediated efflux versus zosuquidar.3HCl plasma concentration and simulated median (and 95% population prediction interval) pharmacokinetic/pharmaco- dynamic profiles. **b** Median pharmacodynamic profiles following administration of zosuquidar.3HCl as a 6-h infusion at 200 mg/m^2 (*dashed-dotted line*) or 300 mg/m^2 (*dashed line*) or as a 3-h infusion at 400 mg/m^2 (*solid line*)

Table 3 Daunorubicin pharmacokinetic parameters in the presence and absence of zosuquidar.3HCl (*V1*, *V2*, *V3* volumes of distribution; *Q2*, *Q3* intercompartmental clearances; *SEE* standard error on the estimate; *NE* not estimated)

Parameter	Population mean estimate (%SEE)	Interpatient variability estimate (%) (%SEE)
Daunorubicin CL (l/h)	168 (11.5)	36.1 (40.5)
Decrease in CL in the presence of zosuquidar.3HCl (%)	10.6 (29.2)	
V1 (l)	24.8 (30.8)	103 (46.6)
Q2 (l/h)	83.4 (24.1)	63.3 (49.4)
V2 (l)	869 (39.6)	NE
Q3 (l/h)	86.3 (20.4)	NE
V3 (l)	98.6 (18.7)	NE
Covariance CL-V1 (%)		57.8 (48.2)
Covariance CL-Q2 (%)		46.8 (44.7)
Covariance V1-Q2 (%)		78.8 (49.8)
Proportional error (SE %)	29.4% (38.6)	

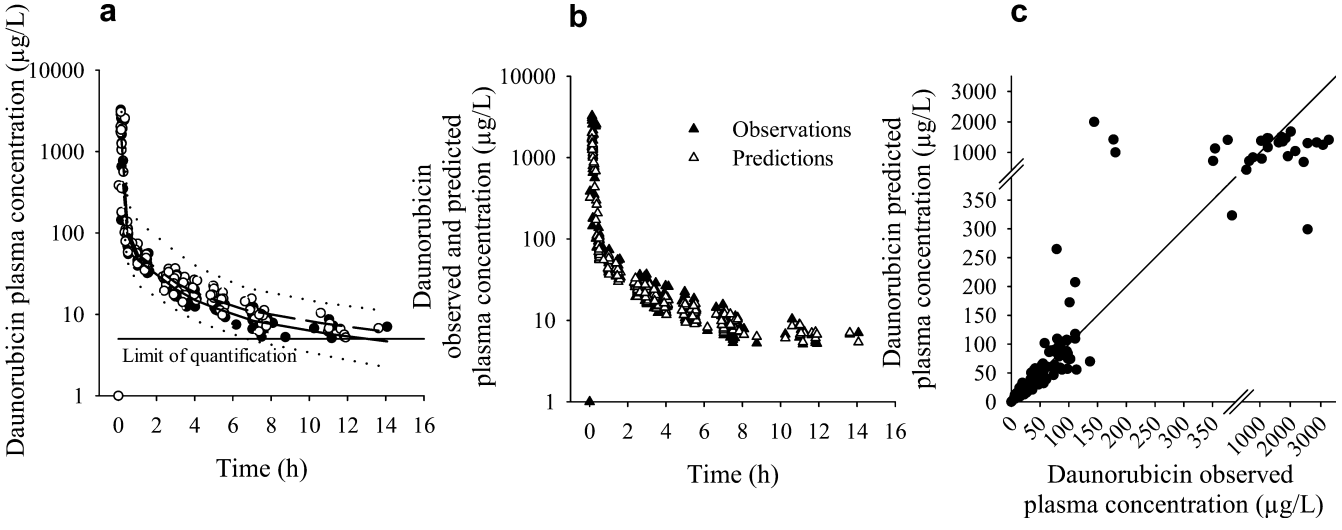

Fig. 4 **a** Daunorubicin observed and median simulated (1000 Monte-Carlo simulations) pharmacokinetic profiles in the absence (*closed circles*, *solid line*) and the presence (*open circles*, *dashed line*) of zosuquidar.3HCl. The *dotted lines* represent the 95% population prediction interval. **b**Daunorubicin observed and predicted pharmacokinetic profiles. **c** Daunorubicin predicted versus observed plasma concentrations

showed a significant increase in DAUNOL AUC in the presence of zosuquidar.3HCl. A structural change in the model, described in Fig. 5, was required to take into account these differences. The goodness of fit plots and apparent DAUNOL pharmacokinetic parameters are presented in Fig. 6 and Table 5, respectively.

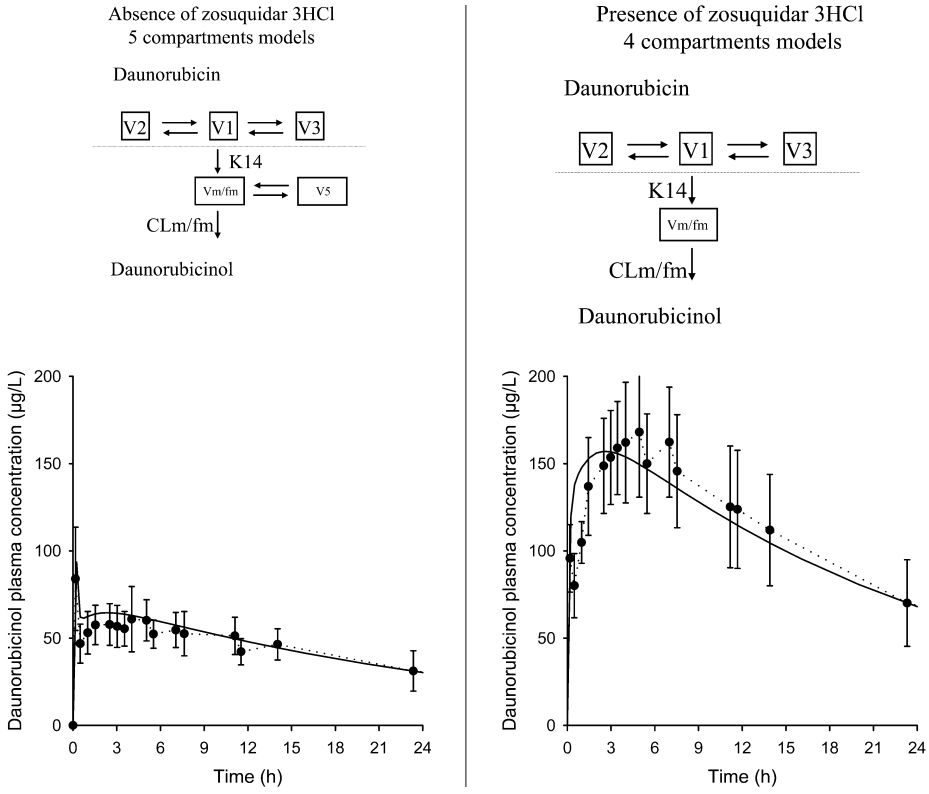

Fig. 5 Daunorubicinol mean ± SD observed and mean simulated pharmacokinetic profiles in the absence (*left*) and presence (*right*) of zosuquidar.3HCl and schematic representation of the corresponding daunorubicin-daunorubicinol pharmacokinetic models

Table 4 Descriptive statistics for daunorubicinol pharmacokinetics in the presence and absence of zosuquidar.3HCl

		Time (h)	Concentration (µg/l)
Absence of zosuquidar.3HCl	First peak		
	Median (range)	0.167	77.05 (42.26–130.50)
	20th percentile		60.22
	Trough		
	Median (range)	0.5	46.30 (29.99–72.20)
	80th percentile		51.23
	Second peak		
	Median (range)	4.5 (1.02–11.03)	61.85 (45.74–102.17)
	20th percentile		52.28
Presence of zosuquidar.3HCl	First peak		
	Median (range)	0.167	89.78 (60.54–138.23)
	20th percentile		85.8
	Trough		
	Median (range)	0.5	84.47 (47.24–111.74)
	80th percentile		91.12
	Second peak		
	Median (range)	4.23 (2.63–10.6)	164.14 (122.83–225.92)
	20th percentile		138.0

Decreases of 55% and 63% in DAUNOL apparent clearance and steady-state volume of distribution, respectively, were observed in the presence of zosuquidar.3HCl. This corresponds to an increase from 7366 to 16370 µg·h/l in DAUNOL AUC following a 400 mg zosuquidar.3HCl dose.

The DAUN-DAUNOL pharmacokinetic model parameters were estimated with reasonable precision and the posterior predictive check (1000 Monte-Carlo simulation) showed that the model predicted the data with no bias.

Discussion

Pharmacokinetic interactions between P-gp inhibitors and coadministered chemotherapies have resulted in increased toxicity, and this has led to reduced doses of

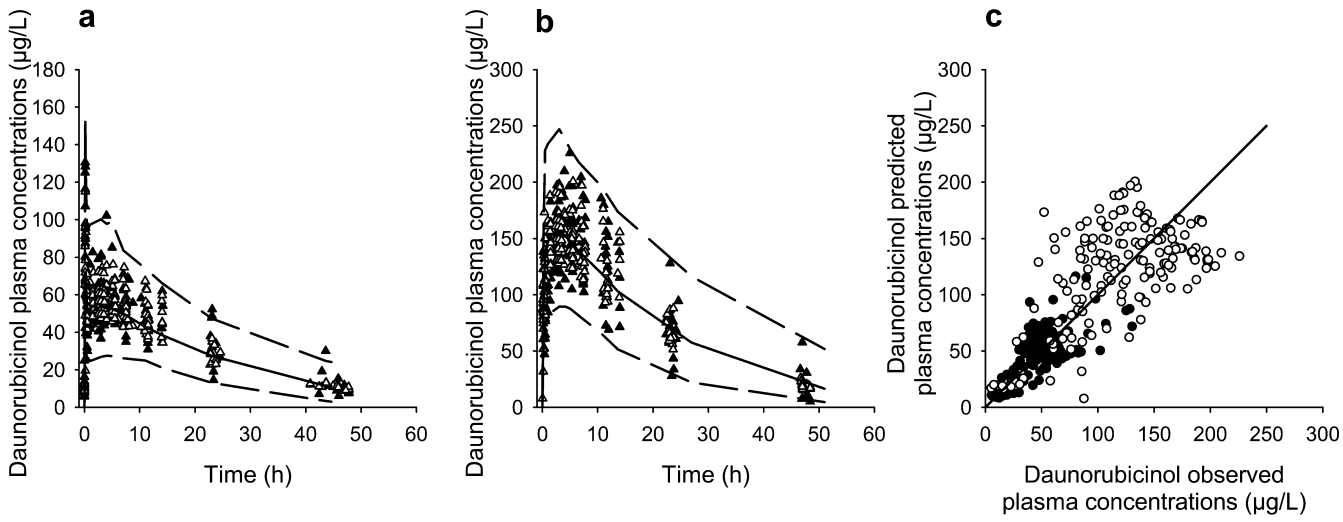

Fig. 6a, b Daunorubicinol observed and simulated pharmacokinetic profiles (median and 95% population prediction interval) in the absence (**a**) and in the presence (**b**) of zosuquidar.3HCl.**c** Daunorubicinol predicted versus observed plasma concentrations in the absence (*closed circles*) and presence (*open circles*) of zosuquidar.3HCl

the chemotherapy when given in combinations [1, 6, 12, 17, 23, 27, 33, 35]. Although the extent of these interactions may have been reduced by increased specificity of the inhibiting agent, some proportion of the interaction may be attributable to the duration of inhibition. An earlier study with zosuquidar.3HCl, a potent third-generation P-gp inhibitor, had established the extent of interactions for a 48-h infusion [5]. In this study, we sought to administer zosuquidar.3HCl as a short infusion in order to examine the impact on coadministered chemotherapy, and to contrast this with the longer infusion schedule above [5]. Key in this determination is an understanding of the degree of the P-gp inhibition together with an appreciation of the pharmacokinetic properties of the molecule itself.

Zosuquidar.3HCl pharmacokinetics in this study were described by a two-compartment model and were characterized by a high plasma clearance (127 l/h) and steady state volume of distribution (539 l) and a rapid distribution and elimination half-life (0.4 and 6.2 h, respectively). Zosuquidar.3HCl pharmacokinetic characteristics illustrate the ability of this P-gp inhibitor to distribute extensively into tissue, and hence into tumours, and to be cleared very rapidly.

The pharmacokinetic/pharmacodynamic model for zosuquidar.3HCl showed a direct reversible sigmoidal E_{max} relationship between the percentage inhibition of Rh123 P-gp-mediated efflux in CD56 cells and zosuquidar.3HCl plasma concentrations. The zosuquidar.3HCl concentrations in excess of the IC_{90} (169.6 µg/l) were readily obtained with this shortened infusion schedule. The dose regimens (3 h and 6 h i.v. infusion of zosuquidar.3HCl) investigated in this study led to maximal P-gp inhibition adequately covering the distribution phase of DAUN. The impact of a short duration of optimal P-gp inhibition (only covering the distribution phase and a brief period of the elimination phase of the coadministered therapy) on the pharmacokinetics of the coadministered therapy could be assessed in this study.

The pharmacokinetics of the anthracycline DAUN have been well documented [18, 23, 24, 25, 26]. The aldoketoreductase enzyme system is responsible for the

Table 5 Daunorubicinol pharmacokinetic parameters in the presence and absence of zosuquidar.3HCl (*CLm/fm* apparent clearance, *Qm/fm* intercompartmental clearance, *Vm/fm* central volume of distribution, *V5/fm* peripheral volume of distribution; *SEE* standard error on the estimate, *NE* not estimated)

Parameter	Population mean estimate (%SEE)	Interpatient variability estimate (%) (%SEE)
CLm/fm (l/h)	54.3 (7.46)	27.3 (36.9)
Interoccasion variability on CLm/fm (%)		15.0 (42.6)
Decrease in CLm/fm in the presence of zosuquidar.3HCl (%)	55.5 (5.22)	
In the absence of zosuquidar.3HCl		
Vm/fm (l)	190 (27.5)	74.9 (39.0)
V5/fm (l)	737 (14.5)	42.5 (63.5)
Qm/fm (l/h)	2050 (17.4)	NE
In the presence of zosuquidar.3HCl		
Vm/fm (l)	343 (5.69)	22.2 (33.8)
V5/fm (l)	NE	NE
Qm/fm (l/h)	NE	NE
Proportional error (SE %)	21.6% (13.2)	

formation of the major metabolite, DAUNOL. It is present in all cells, but particularly white blood cells, red blood cells, liver and kidney. Preclinical and clinical investigations have shown that DAUN is eliminated by biliary excretion. Approximately 60% of the dose is recovered in the bile, of which the metabolite DAUNOL represents the greatest proportion (about 90%) [22]. Limited quantities may also be recovered in the urine (about 10%). Both P-gp and MRP are involved in the excretion of both DAUN and DAUNOL and hence inhibition of P-gp results in pharmacokinetic interactions.

A five-compartment model (Fig. 5) was required to describe DAUN-DAUNOL pharmacokinetics in the absence of zosuquidar.3HCl. The three compartments, corresponding to V1, V2 and V3 of the five compartment model, adequately described the triexponential pharmacokinetics of the parent molecule, DAUN. Two additional compartments corresponding to Vm/fm and V5 were required to explain the two peaks observed in the plasma concentration-time curve for the metabolite, DAUNOL. Although this model is empirical, there may be a plausible mechanistic explanation.

DAUNOL pharmacokinetic profiles in the absence of zosuquidar.3HCl can be interpreted as followed. The rapid and extensive DAUN metabolism begins in the blood cells and leads to a steep rise in DAUNOL plasma concentrations followed by tissue distribution and elimination (decrease in the concentration). Together, these processes lead to the first early DAUNOL peak plasma concentration at 0.17 h. Thereafter, at approximately 0.5 h (time of the trough concentration), the DAUNOL concentrations begin to increase. This is likely to result from the further metabolism of DAUN in the liver. DAUNOL elimination is slower than its formation. This results in DAUNOL distribution from the liver to the plasma. This explains the second peak concentration at 4.5 h which is followed by the predominance of the elimination process over the formation process and the consequent fall in DAUNOL plasma concentrations (Table 4, Fig. 5).

In the presence of zosuquidar.3HCl, DAUNOL pharmacokinetics were described by a four-compartment model, with the three compartments describing the parent pharmacokinetics being unchanged. Zosuquidar.3HCl inhibits the hepatic elimination of both DAUN and DAUNOL by inhibition of P-gp in the biliary system. Consequently, this exacerbates the predominance of formation of metabolite relative to its elimination. This results in two distinct outcomes. First, the DAUNOL peak plasma concentrations are similar or greater in the presence compared to the absence of zosuquidar.3HCl, (in the absence and presence of Zosuquidar.3HCl, DAUNOL median peak plasma concentration of 77.05 (t_{max} 0.17 h) -61.85 μg/l (t_{max} 4.5 h) and 89.78 (t_{max}0.17 h) -(164.14 μg/l (t_{max} 4.5 h), respectively). Second, the increasing DAUNOL plasma concentration due to hepatic metabolism masks any decrease due to tissue distribution from blood cell metabolism. The net effect of this is the disappearance of DAUNOL plasma peak concentration at 0.17 h in the presence of P-gp inhibition (median trough plasma concentration at 0.5 h of 46.30 μg/l and 84.47 μg/l in the absence and the presence of zosuquidar.3HCl, respectively). The distribution compartment that allowed the prediction of the DAUNOL double peak pharmacokinetics was therefore no longer required (Table 4, Fig. 5).

Overall DAUN clearance showed minimal change in the presence of zosuquidar.3HCl (about 10% reduction). However, there was a greater reduction in DAUNOL apparent clearance (about 50%). In this study DAUN was administered sequentially 48 h apart and hence these results need to be considered with caution since it is assumed that both DAUN and DAUNOL pharmacokinetics are time- and concentration-independent. This, however, may not be a valid assumption since aldoketoreductase activity is likely to be inducible [15, 16]. Therefore, in the present study, the increase observed in DAUNOL AUC could plausibly result from both an increase in its formation due to enzyme induction and a decrease in its elimination due to inhibition of P-gp. A randomized study would be necessary to delineate the true impact of zosuquidar.3HCl on DAUN and DAUNOL pharmacokinetics from a nonlinear phenomenon such as enzyme induction.

The impact of the length of infusion (6 h or 3 h) of zosuquidar.3HCl on the pharmacokinetic interaction with DAUN CL was investigated. However, the approximate difference in DAUN clearance (15% versus 8% reduction) following the 6-h and 3-h infusion, respectively, was not considered statistically significant at the *P* value of 0.1. This was largely due to the small number of subjects in this study (seven and nine subjects for the 6-h and 3-h infusion schemes, respectively). The magnitude of the reduction in anthracycline clearance contrasts with that observed in a previous study [5] with a longer schedule of administration of zosuquidar.3HCl where the difference in DOX clearance was 25%. The effects of the duration of infusion on the pharmacokinetics of the metabolites (DOXOL and DAUNOL) in the two studies, respectively, could not be compared for the reasons outlined above with respect to the study design and the DAUNOL accumulation. The length of infusion could also influence the t_{max} of DAUNOL. This was previously observed for DOXOL for which the t_{max} was delayed with a prolonged concomitant zosuquidar.3HCl infusion of 24 h. In the study reported here, the t_{max} of the second peak in the absence of zosuquidar.3HCl approximates to the duration of the zosuquidar.3HCl infusion and hence an impact of DAUNOL t_{max} was not observed.

A decrease in the apparent volume of distribution of DAUNOL from 927 l (190 + 737 l) in the absence of zosuquidar.3HCl to 343 l in its presence is noted. The mechanism responsible for this decrease remains unclear. Such a decrease with P-gp inhibition for a variety of P-gp substrates has been reported previously both for zosuquidar.3HCl and for other P-gp inhibitors [5, 34, 39].

In conclusion, a short i.v. infusion schedule for zosuquidar.3HCl is feasible and produces maximal P-gp inhibition, but reduced pharmacokinetic interactions. The pharmacokinetic characteristics of zosuquidar.3HCl allow flexible infusion schedules designed to optimize the duration of maximal P-gp inhibition whilst minimizing the impact on elimination of the oncolytic and hence toxicity. A randomized study is required to validate the models from this study, to assess the true impact of zosuquidar.3HCl on DAUN-DAUNOL pharmacokinetics and to determine the benefit in clinical outcome from such an approach. Such a study is already ongoing in collaboration with the Eastern Cooperative Oncology Group. This study will enrol more than 400 patients greater than 60 years of age with newly diagnosed AML or refractory anaemia with excess blasts (RAEB). Patients will be randomized to receive conventional evolution and post-remission therapy with and without zosuquidar.3HCl.

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