Cancer Chemother Pharmacol (2005) 55: 488–496
DOI 10.1007/s00280-004-0900-4

ORIGINAL ARTICLE

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Pharmacokinetics of low-dose doxorubicin and metabolites in patients with AIDS-related Kaposi sarcoma

Received: 10 May 2004 / Accepted: 12 July 2004 / Published online: 23 February 2005

Abstract *Purpose*: Systemic chemotherapy is the treatment of choice for AIDS-related advanced Kaposi sarcoma. One principal schedule combines adriamycin (doxorubicin), bleomycin, and vincristine (ABV). We analysed the plasma concentrations of low-dose doxorubicin (Dx) and its metabolites doxorubicinol, 7-deoxydoxorubicinone, doxorubicinone, doxorubicinolone, and 7-deoxydoxorubicinolone in AIDS-patients to define patient-group and dose-specific pharmacokinetic parameters. *Materials and methods*: A previously described high-performance liquid chromatographic (HPLC) method and a population approach with non-linear mixed effects modelling (NONMEM) were used for analysis and subsequent modelling of the time–concentration data of low-dose Dx and metabolites in seven patients with AIDS-related advanced Kaposi sarcoma. Patients received Dx 20 mg m^{-2}, bleomycin 15 U m^{-2} and vincristine 2 mg as a 30-min intravenous infusion each. Blood samples were collected up to 72 h after the start of Dx treatment. WinNonlin software version 4.1 was used for non-compartmental analysis and NONMEM software version V for compartmental analysis. Covariate analysis was performed for various clinical and laboratory parameters. *Results*: Non-compartmental analysis yielded an area under the plasma concentration–time curve (AUC) for Dx of 566 μg h L^{-1}, a maximum plasma concentration (c_{max}) of 599 μg L^{-1} and an elimination half-life ($t_{1/2}$) of 30.8 h. Compartmental analysis resulted in a two-compartment model with first-order elimination, which best fitted the concentration–time data. Model estimate for Dx clearance was 61.8 L h^{-1}, for intercompartmental clearance (Q) 112 L h^{-1}, for the volume of the central compartment (V_1) 23.3 L, and for the volume of the peripheral compartment (V_2) 1,130 L. Metabolite data could adequately be estimated by NONMEM using single-compartment models. Graphical plots of residuals versus time for all metabolites yielded no evidence of non-linear pharmacokinetic behaviour. Laboratory parameters of liver and renal function were all in the normal range and their inclusion in the pharmacokinetic model did not improve data fit. A final jack-knife analysis was performed. *Conclusions*: Concentration–time data for low-dose Dx and metabolites in the ABV-regimen are best described by a two-compartment model with first-order elimination. The results confirm that the aglycones doxorubicinone, 7-deoxydoxorubicinone, and doxorubicinolone can be reliably detected in the studied patient group and implemented into a common metabolic model. Model estimates suggest that pharmacokinetic parameters are similar for low-dose Dx and higher-dosed Dx. As the role of the aglycones is still poorly understood, despite their potential clinical relevance, their analysis should be implemented in future pharmacokinetic and pharmacodynamic studies of Dx.

Keywords Doxorubicin · Pharmacokinetics · Non-linear mixed effects modelling · Population PK modelling · Kaposi sarcoma · Low-dose chemotherapy

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Introduction

AIDS-related Kaposi sarcoma is a tumour of vascular origin, highly correlated with advanced immunosuppression and concurrent infection with human herpesvirus-8. While the incidence of AIDS-related Kaposi sarcoma has decreased in the US and Europe as a consequence of highly-active antiretroviral therapy (HAART), it is dramatically increasing in Africa as a consequence of spreading infection with HIV and possibly also human herpesvirus-8 [1]. No cure is available for Kaposi sarcoma and treatment goals are therefore palliative. Systemic chemotherapy is the treatment of choice for Kaposi sarcoma that is extensive or disseminated to visceral organs. Besides paclitaxel and the newer liposomal anthracyclines, ABV (adriamycin, bleomycin, vincristine) is a principal combination chemotherapy for Kaposi sarcoma, combining 2-weekly intravenous adriamycin (doxorubicin) [(40 mg m^{-2} standard-dose or 20 mg m^{-2} low-dose, as used in this study)], bleomycin 10–15 U m^{-2} and vincristine 1–2 mg, with response rates between 25% and 88% [2]. Low doses of doxorubicin (Dx) have been implemented in the ABV regimen to diminish immunosuppression associated with dose-dense Dx schedules.

Dx is a widely used drug in oncology. It inhibits cell growth by DNA intercalation, generation of free radicals, interaction with cellular membranes and inhibition of topoisomerase II [3]. Metabolic pathways of Dx are detailed in Fig. 1. Dx undergoes reduction of the ketone on carbon 13 to build the secondary alcohol doxorubicinol (Dx-ol). A unique feature of Dx metabolism is the formation of aglycones from Dx and Dx-ol either by reductive removal of the C7-linked daunosamine sugar group via a semi-quinone intermediate and subsequent protonation of the C7-aglycone radical to build 7-deoxydoxorubicinone (7-deoxyDx-one) from Dx and 7-deoxydoxorubicinolone (7-deoxyDx-olone) from Dx-ol, or by acid-catalysed hydrolysis of the glycosidic bond, eliminating the sugar component to leave doxorubicinone (Dx-one) from Dx and doxorubicinolone (Dx-olone) from Dx-ol (Fig. 1) [4, 5].

The pharmacokinetics of Dx are most commonly described by two-compartment [6–10] or three-compartment [7, 8, 11, 12] models, depending on the length of intravenous drug infusion and the number and timing of blood samples. Although Dx and Dx-ol are cytotoxic, this is less evident for the aglycones, because their clinical effects are poorly understood. Aglycones, however, may be clinically important metabolites, because they have been reported to be responsible for acute cardiac toxicity by causing oxidative stress [13]. Difficulties with analysing low plasma concentrations of the aglycones and poor understanding of the metabolic pathways of Dx have mainly hampered clinical exploration of the aglycones. We have implemented a previously described high-performance liquid chromatographic (HPLC)

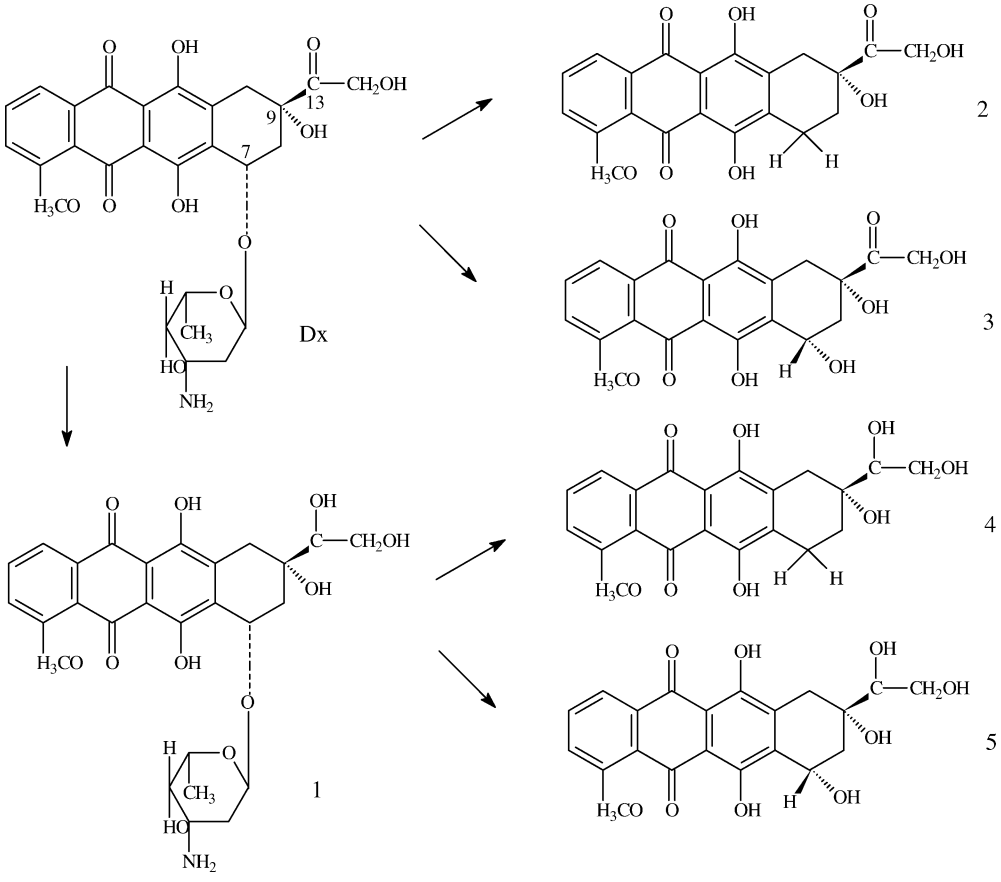

Fig. 1 Chemical structure of Dx and its metabolites. Dx doxorubicin, (*1*) doxorubicinol (Dx-ol), (*2*) 7-deoxydoxorubicinone (7-deoxyDx-one), (*3*) doxorubicinone (Dx-one), (*4*) 7-deoxydoxorubicinolone (7-deoxyDx-olone), (*5*) doxorubicinolone (Dx-olone)

method and a population approach with non-linear mixed effects modelling (NONMEM) to study the pharmacokinetics of low-dose Dx and metabolites in patients with AIDS-related Kaposi sarcoma.

Experimental and methods

Patients and treatment

Seven patients with HIV-infection stage C3 (i.e. AIDS-indicator conditions including Kaposi sarcoma and a CD4 cell count $< 200\ \mu L^{-1}$) and advanced Kaposi sarcoma were treated with systemic Dx 20 mg m^{-2} (36.8 μmol m^{-2}), bleomycin 15 U m^{-2} and vincristine 2 mg within a clinical research program focusing on the metabolism and the pharmacokinetics of Dx in patients with AIDS (see also Table 1). None of the patients had received previous chemotherapy for Kaposi sarcoma. Granulocyte-macrophage colony-stimulating factor (GM-CSF) was used if deemed necessary by the treating physicians. Dx, bleomycin, and vincristine were dissolved in 100 mL 0.9% sodium chloride infusion and given, in this order, as a 30-min intravenous infusion each.

Sample procedure, liquid chromatography and analytical precision

Blood samples were collected from an intravenous cannula in the arm contralateral to that receiving chemotherapy at specific times up to 72 h post-infusion. All patients were sampled at their first ABV chemotherapy cycle. Target sampling times were 0.08, 0.25, 0.42, 0.67, 1, 1.5, 2, 4, 6, 12, 24, 48 and 72 h after the start of Dx infusion. Blood samples were collected in EDTA tubes and plasma was immediately isolated by centrifugation and stored at −30°C before analysis. Concentrations of Dx, Dx-ol, Dx-one, 7-deoxyDx-one and Dx-olone were quantitatively assessed by HPLC as described previously [14]. The lower limit of quantitation was 1 ng mL^{-1} for Dx and Dx-ol and 0.5 ng mL^{-1} for the aglycones. Within and between-day imprecision was less than 2.40% for Dx, less than 2.15% for Dx-ol, less than 1.63% for Dx-one, less than 1.62% for 7-deoxyDx-one, and less than 1.70% for Dx-olone. Accuracy ranged between 91% and 107% for all compounds. Details concerning chemicals, sample preparation and chromatographic equipment have been published elsewhere [14].

Table 1 Patient characteristics

Parameter	Units	Normal range	Mean (range)
Age	Years		43.4 (29–53)
CD4-count	cells μL^{-1}	700–1,100	62 (20–120)
Weight	kg		67.2 (61–77)
Number of chemotherapy cycles			5.8 (1–11)
Aspartate aminotransferase (ASAT)	U L^{-1}	≤ 40	16 (11–28)
Alanine aminotransferase (ALAT)	U L^{-1}	≤ 45	14 (10–18)
Total bilirubin	μmol L^{-1}	< 16	8 (6–16)
Serum creatinine	μmol L^{-1}	50–105	89 (82–102)
Serum albumin	g L^{-1}	35–50	33 (27–42)
Alkaline phosphatase (AP)	U L^{-1}	40–120	93 (79–106)
Gammaglutamyl transpeptidase (GGT)	U L^{-1}	≤ 50	19 (15–24)

Non-compartmental analysis

WinNonlin software version 4.1 (Pharsight Corporation, Mountain View, CA, USA) was used in a first approach for non-compartmental analysis of the concentration–time data to obtain the area under the plasma concentration–time curves from time 0 to ∞ (AUC_{inf}), elimination half-life ($t_{1/2}$), maximum plasma concentration (c_{max}), and time to c_{max} (t_{max}) of Dx and its metabolites. The log–linear trapezoidal rule was used to estimate the respective AUC_{inf} values, and the AUC_{inf} ratios of the metabolites to the parent compound Dx were calculated to determine exposure to any metabolite compared with the parent drug.

Compartmental analysis

Compartmental analysis of concentration–time data for Dx and its metabolites was performed using the NONMEM program version V (double precision, level 1.1) [15]. NONMEM uses a maximum likelihood criterion to simultaneously estimate population median values of fixed-effects parameters (e.g. clearance, volume of distribution) and values of the random-effects parameters (i.e. inter-individual and intra-individual or residual variability). The first-order (FO) method with logarithmic transformation of all drug-concentration data was used throughout. The minimum value of objective function, as calculated by NONMEM, was used to compare different models, for example in the covariate testing procedure. The difference in objective function between two nested models is approximately chi-square distributed with one degree of freedom. Standard errors for all parameters were calculated using the COVARIANCE option of NONMEM and individual Bayesian pharmacokinetic parameters were obtained with the POSTHOC option [15]. The S-plus (MathSoft, Seattle, USA) based model-building aid Xpose 3.0 was used for graphical processing [16]. An open two-compartment model with first-order elimination was used to describe the data. Both one-compartment and three-compartment models were tested. Inter-individual variability was estimated using a proportional error model. For example,

inter-individual variability in clearance was estimated using $CL/F_i = \theta\exp(\eta_i)$, in which CL/F_i represents the apparent clearance of the ith individual, θ is the typical value of CL/F in the population, and η_i is the inter-individual random effect with mean 0 and variance ω^2. Intra-individual or residual variability was modelled as $\log(C_{ij}) = \log(\hat{C}_{ij}) + \varepsilon_{ij}$, in which C_{ij} and $\hat{C}_{ij}$ are the jth measured and model predicted drug concentrations of the ith individual, respectively, and ϵ_{ij} is the residual random error with mean 0 and variance σ^2. A two-stage approach was chosen for population modelling. A first sub-model included concentration–time data of Dx to estimate Dx clearance (CL), intercompartimental clearance (Q), and volume of distribution of the central (V_1) and peripheral (V_2) compartments by applying a two-compartment model with first-order elimination from the central compartment and the NONMEM subroutine ADVAN3. Individual estimates of CL, Q, V_1 and V_2 from the POSTHOC option of the first sub-model were subsequently included in a second sub-model comprising the concentration–time data for all metabolites. NONMEM subroutine ADVAN5 was used for the second sub-model. Each metabolite of Dx was assigned to a separate compartment, so that compartment three comprised concentration–time data of Dx-one, compartment four those of 7-deoxyDx-one, compartment five those of Dx-ol, and compartment six those of 7-deoxyDx-olone. For all metabolites, the fraction of the initial Dx dose converted to the respective metabolite divided by the volume of distribution of the respective metabolite (FM x) and the elimination rate constant (Kx) were subsequently estimated. The elimination rate constant for Dx was divided into formation rate constants of the metabolites ($K13$, $K14$, $K15$) and residual elimination ($K10$) as follows:

$$K10 = (1 - \mathrm{FM3} - \mathrm{FM4} - \mathrm{FM5})(\mathrm{CL}/V_1)$$
$$[\text{elimination rate constant of Dx}]$$
$$K13 = \mathrm{FM3}(\mathrm{CL}/V_1)$$
$$[\text{formation rate constant of Dx - one}]$$
$$K14 = \mathrm{FM4}(\mathrm{CL}/V_1)$$
$$[\text{formation rate constant of 7 - deoxyDx - one}]$$
$$K15 = \mathrm{FM5}(\mathrm{CL}/V_1)$$
$$[\text{formation rate constant of Dx - ol}]$$

The elimination rate constants of Dx-one ($K30$), 7-deoxyDx-one ($K40$), Dx-ol ($K56$) and 7-deoxyDx-olone ($K60$) were individually estimated by NONMEM, as were the ratios of metabolite fraction/volume of distribution for Dx-one ($FM3$), 7-deoxyDx-one ($FM4$), and Dx-ol ($FM5$).

To establish possible correlations between the pharmacokinetic data and patient characteristics, the following data were collected at baseline and subjected to covariate analysis: patient weight, comedication, serum creatinine, liver function as assessed by alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), and serum total bilirubin. At the time of data analysis, comedication was not included as a covariate because it could not be retrieved reliably according to the precise point in time and duration of drug intake. A covariate was considered statistically significant when inclusion of the covariate was associated with a decrease in the minimum value of objective function of at least 3.8 points (P-value ≤ 0.05). A subsequent backward elimination analysis was planned and a covariate was retained in the model when the influence of the parameter was statistically significant, resulting in a 7-point drop in the minimum value of objective function ($P \leq 0.01$). A final jack-knife analysis was executed by fitting the final model to the population minus one individual. This was subsequently repeated until every individual had been excluded from the full model, resulting in seven different analyses (in accordance with 7 patients). Unbalanced results are expected if the parameter estimates of the full model are highly determined by a single individual.

Results

Patient characteristics

Data from seven male HIV patients were available and the respective clinical characteristics are detailed in Table 1. All patients had HIV-infection stage C3 with a mean CD4-count of 62 cells μL^{-1} (range 20–120 μL^{-1}). Mean patient age was 43.4 years (range 29–53 years) and mean patient weight was 67.2 kg

Table 2 Non-compartmental analysis

Metabolite	AUC_{inf} mean ($\mu g\ h\ L^{-1}$) (SD)	$t_{1/2}$ terminal mean (h) (SD)	c_{max} mean ($\mu g\ L^{-1}$) (SD)	t_{max} mean (h) (SD)
Doxorubicin (Dx)	566 (103)	30.8 (18.4)	599 (110)	0.30 (0.06)
Doxorubicinol (Dx-ol)	298 (91.1)	27.8 (10.6)	30.5 (2.45)	0.56 (0.07)
Doxorubicinone (Dx-one)	NE	NE	8.43 (3.24)	0.36 (0.16)
7-DeoxyDx-one	NE	NE	4.84 (1.51)	0.34 (0.16)
Doxorubicinolone (Dx-olone)	NE	NE	1.79 (0.18)	0.61 (0.11)

NE Not estimated

(range 61–77 kg). Six patients were Caucasian and one patient was of Asian origin. Patients received a mean of 5.8 cycles of ABV chemotherapy (range 1–11 cycles). Dx, Dx-ol, and 7-deoxyDx-one were detected in all patients. Dx-one and Dx-olone were not detectable in two patients each. 7-DeoxyDx-olone was not detected in the studied patient group. The final database consisted of 103 Dx concentration–time points, 101 Dx-ol concentration–time points, 44 Dx-one concentration–time points, 66 Dx-olone concentration–time points and 56 7-deoxyDx-one concentration–time points.

Non-compartmental analysis

Estimates for AUC_{inf}, $t_{1/2}$, c_{max}, and t_{max} as derived from non-compartmental analysis are summarised in Table 2. Non-linear pharmacokinetic behaviour was not observed for Dx nor for any of its metabolites. Mean AUC_{inf} for Dx was roughly double that of Dx-ol (566 μg h L^{-1} vs 298 μg h L^{-1}). Mean $t_{1/2}$ was in the same range for Dx (30.8 h) as for Dx-ol (27.8 h). AUC_{inf} and $t_{1/2}$ could not be determined for the aglycones, because of the limited set of concentration–time data. Mean c_{max} was about 20 times higher for Dx than for

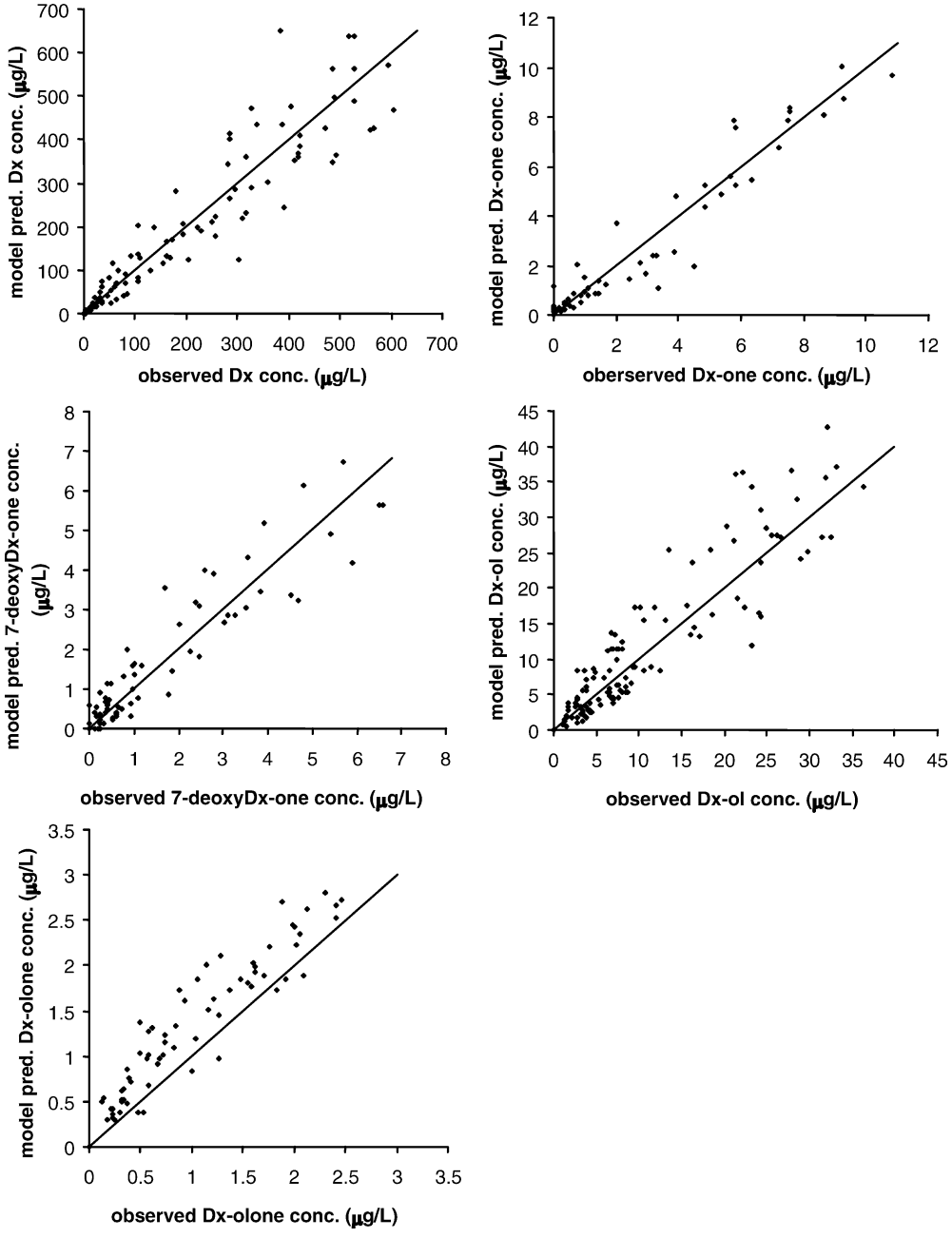

Fig. 2 Model-predicted concentrations (PREDs) versus observed concentrations of doxorubicin (Dx), doxorubicinone (Dx-one), 7-deoxy-doxorubicinone (7-deoxyDx-one), doxorubicinol (Dx-ol), and doxorubicinolone (Dx-olone)

Dx-ol, whereas mean c_{max} was below 8 μg L^{-1} for the aglycones. For Dx-ol, time to reach c_{max} (t_{max}) was almost double that for Dx (0.56 h vs 0.30 h). C_{max} and t_{max} of the 7-aglycones are outlined in Table 2.

Compartmental analysis

NONMEM analysis resulted in a two-compartment model with first-order elimination, which best fitted the set of observations of Dx. A one-compartment model resulted in a significantly worse fit and a three-compartment model did not improve the fit either. Estimated Dx CL was 61.8 L h^{-1} with an inter-individual variability of 14.3% (Table 3). The estimate for inter-compartimental clearance (Q) was 112 L h^{-1} (inter-individual variability 19.4%), for the volume of the central compartment (V_1) 23.3 L (inter-individual variability 44.7%), and for the volume of the peripheral compartment (V_2) 1,130 L (inter-individual variability 25.5%). Single compartment models were used for analysis of the metabolite data, in which each metabolite was assigned to a respective compartment. This resulted in an adequate data-fit except for Dx-olone. This is further outlined in Fig. 2, in which observed metabolite concentrations are separately plotted against model-predicted concentrations for each metabolite. The final population model tends to overestimate plasma concentrations of Dx-olone as can be seen in the respective plot in Fig. 2. Because of the limited amount of concentration–time data, however, further model refinement was not possible.

Laboratory parameters for liver and renal function including ALAT, ASAT, serum total bilirubin and serum creatinine were all in the normal range (Table 1). Their inclusion in the pharmacokinetic model did not improve data fit, because none of the tested covariates led to a significant decrease in the minimum value of the objective function of at least 3.8 points. Neither was patient weight identified as a significant covariate.

The concentration–time points of Dx and metabolites and model-derived individual predictions (IPREDs) are illustrated for a typical patient in Figs. 3 and 4. It can be seen that individual model predictions adequately fit the observed concentration–time data for this patient. Final estimates of NONMEM analysis are detailed in Table 3, including standard deviations and intra-individual variability. Results from the jack-knife analysis are also detailed in Table 3, on the right side, with median and range. Median values are in the same range as the

Table 3 Population pharmacokinetic data. CV coefficient of variation (CV% standard deviation divided by the mean and multiplied by 100), K elimination rate constant, η interindividual variability, ϵ residual random variability, NE not estimated

Pharmacokinetic parameter	Full data set			Jack-knife validation	
	Units	Estimate	CV%	Median	Range
CL (Dx)	L h^{-1}	61.8	10.2	62.3	57.2–66.4
Q (central-peripheral)	L h^{-1}	112	27.6	117	84–119
V_1 (Dx, central)	L	23.3	7.3	23.3	21.9–24.9
V_2 (Dx, peripheral)	L	1,130	18.0	1,210	970–1,230
Fraction of metabolite 3 (Dx-one)/V_3	L^{-1}	9.33×10^{-3}	27.7	9.36×10^{-3}	7.38×10^{-3}–1.06×10^{-2}
Fraction of metabolite 4 (7-deoxyDx-one)/V_4	L^{-1}	3.44×10^{-3}	35.7	3.53×10^{-3}	2.61×10^{-3}–4.38×10^{-3}
Fraction of metabolite 5 (Dx-ol)/V_5	L^{-1}	7.33×10^{-3}	15.7	7.63×10^{-3}	6.52×10^{-3}–9.58×10^{-3}
K_{30} (Dx-one)	h^{-1}	22.2	37.0	22.2	17.8–35.0
K_{40} (7-deoxyDx-one)	h^{-1}	7.22	15.1	7.35	6.24–7.77
K_{56} (Dx-ol)	h^{-1}	2.18	13.5	2.19	2.14–2.69
K_{60} (7-deoxyDx-olone)	h^{-1}	62.6	20.4	59.9	32.2–75.6
Interindividual variability					
η CL (Dx)	%	14.3	13.3[a]	13.2	10.7–20.7
η Q (central-peripheral)	%	19.4	25.5[a]	21.6	16.3–23.8
η V_1 (Dx, central)	%	44.7	51.9[a]	45.1	21.9–53.8
η V_2 (Dx, peripheral)	%	25.5	19.7[a]	26.8	11.1–29.4
η fraction of metabolite 3 (Dx-one)		NE			
η fraction of metabolite 4 (7-deoxyDx-one)	%	94.4	67.8[a]	97.2	76.4–105
η fraction of metabolite 5 (Dx-ol)	%	35.4	20.8[a]	36.1	18.5–42.7
η K_{30}	%	69.3	44.8[a]	69.2	43.5–74.8
η K_{40}	%	130	98.9[a]	137	129–157
η K_{56}		NE			
η K_{60}	%	37.9	91.8[a]	32.6	18.6–50.7
Residual variability					
ϵ Dx	%	34.6	16.7[a]	34.6	25.7–37.4
ϵ Dx-ol	%	36.2	25.6[a]	44.7	34.4–49.8
ϵ Dx-one	%	22.7	23.2[a]	23.3	19.5–24.4
ϵ 7-deoxyDx-one	%	22.9	12.3[a]	29.7	25.1–31.4
ϵ Dx-olone	%	18.8	11.3[a]	22.9	12.9–34.9

[a]CVs refer to the estimated interindividual and residual variances

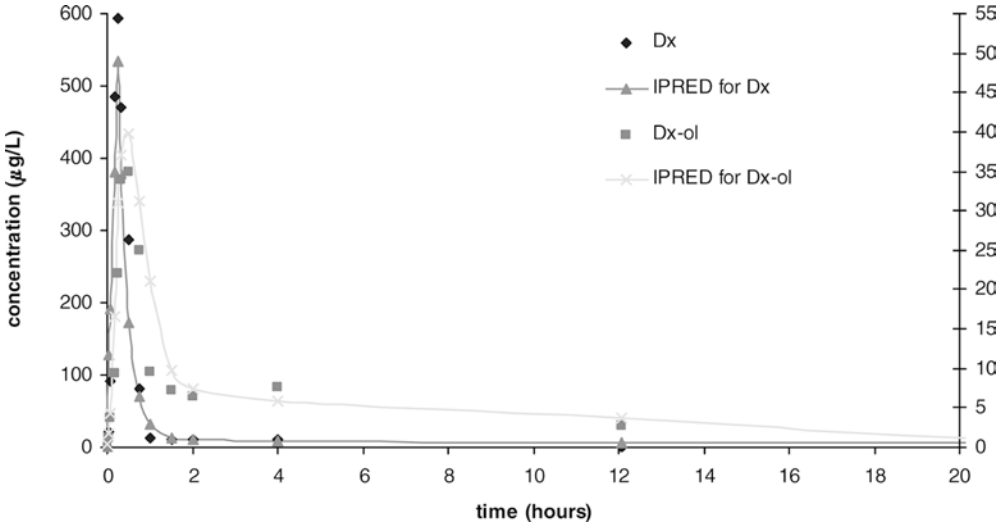

Fig. 3 Concentration–time points for Dx and Dx-ol compared with individual model predictions (IPRED) for patient number 1. Concentrations on the *left* *x*-axis for Dx, on the *right* *x*-axis for Dx-ol

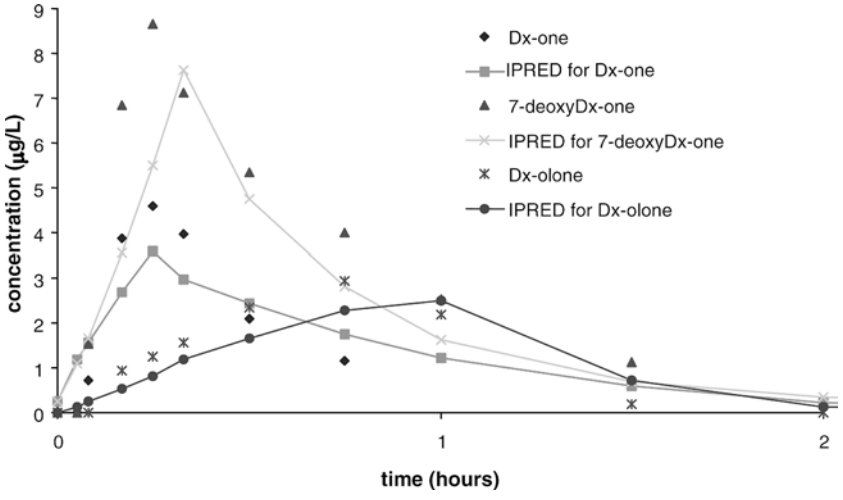

Fig. 4 Concentration–time points for the aglycones Dx-one, 7-deoxyDx-one and Dx-olone compared to the individual model predictions (IPRED) for patient number 1

respective model estimates for every analysed parameter. Values of AUC_{inf} for the aglycones were derived from individual Bayesian estimates of NONMEM analysis, because they could not be reliably estimated by the non-compartmental analysis (see Table 4). The AUC-ratio of Dx-ol and Dx was 0.47 in our model, in accordance with literature data [11]. Furthermore, we found an AUC-ratio of 7-deoxyDx-one/Dx of 0.005, less than that reported by Cummings and colleagues (0.01–0.05) [17].

Discussion

This is the first report on the population pharmacokinetics of low-dose Dx and metabolites in AIDS-patients. The difficulty of detecting the various metabolites of Dx has long hampered detailed analysis of its biotransformation and rendered pharmacokinetic and pharmacodynamic assessment of the aglycones impossible. A sensitive HPLC method has been introduced at our institute and recovery was subsequently shown to be high for Dx, Dx-ol, and the aglycones [14]. Low-dose Dx is of significant clinical importance, especially in immunocompromised patients, for example AIDS-patients [18]. The application of low-dose Dx schedules can significantly reduce drug-related side-effects, particularly cumulative cardiac injury and immunosuppression, and concurrently preserve clinical activity [19–21].

We studied a homogenous group of patients with HIV-infection stage C3 receiving their first ABV chemotherapy cycle for advanced Kaposi sarcoma. There were no indications of impaired liver function, because neither liver transaminases nor total bilirubin were significantly elevated. Dx, Dx-ol and the aglycones Dx-one, 7-deoxyDx-one and Dx-olone were found in all patients, except for Dx-one and Dx-olone, which could not be detected in two patients, and 7-deoxyDx-olone, which was not detected in the group as a whole. In comparison, aglycones were found in 60% of patients in a study by Cummings and colleagues [17]. Our results therefore suggest that the aglycones are reliably detectable metabolites of Dx and Dx-ol, as has also been shown by

Table 4 Estimates of AUCs of Dx-one, 7-deoxyDx-one and Dx-olone derived from Bayesian estimates

Pharmacokinetic parameter	Units	Model estimate	Range
AUC Dx-one	μg h L^{-1}	16.4	6.5–21.1
AUC 7-deoxyDx-one	μg h L^{-1}	6.8	1.3–12.7
AUC Dx-olone	μg h L^{-1}	9.3	4.6–16.9

Mross and colleagues [8]. The concentration–time data of low-dose Dx and metabolites, as given to AIDS-patients with advanced Kaposi sarcoma, could be reliably implemented in a two-compartment model with first-order elimination (Table 3). The estimate of Dx clearance (61.8 L h^{-1}) is in accordance with clearance data of standard-dose Dx ranging from 24 L h^{-1} to 73 L h^{-1} [22, 23]. Similarly, estimates for Dx $t_{1/2}$ (30.8 h) and V_2 (16.8 L kg^{-1}) are in accordance to those reported for standard-dose Dx [11, 22]. Estimates for Q and V_2 suggest very rapid and extensive distribution, a well-known characteristic of Dx [8, 11]. Graphical plots of residuals versus time for all metabolites yielded no evidence of non-linear pharmacokinetic behaviour. However, to confirm linear pharmacokinetics, further studies implementing various Dx dosages are necessary. Model estimates for the pharmacokinetic parameters of 7-deoxyDx-one (i.e. fraction of 7-deoxyDx-one/V_4 and K_{40}) were characterised by a significant inter-individual variability, similar to that found in a study by Andersen and colleagues [6]. The reason for the highly variable metabolism of 7-deoxyDx-one is unclear. In general, it is likely that the actual estimates of inter-individual variability underestimate the true variability, because the studied patient population was homogenous and small. The reasons for the low AUC ratio of 7-deoxyDx-one/Dx compared with data from Cummings and colleagues [17] are unclear. It would be reasonable to suggest concomitant subclinical liver dysfunction that impaired metabolic formation of 7-deoxyDx-one in this patient group. This however seems unlikely because laboratory data do not reveal liver impairment. The results from the validating Jack-knife analysis (Table 3) confirm that NONMEM-derived model estimates are reliable and not disproportionately dependent on data from a single individual. The lack of identification of significant covariates such as patient weight and parameters of organ function is probably because tested laboratory parameters were all within the normal range and thus unlikely to add to the goodness of data fit. Furthermore, the possible influence of various comedications on Dx pharmacokinetics was difficult to assess and may even be complicated by non-reported illegal drug abuse. Drawbacks of our population model are the relatively sparse concentration–time data set with the associated risk of overparameterization when using compartmental modelling.

In conclusion, we present herein a population model of low-dose Dx and metabolites in AIDS-patients with advanced Kaposi sarcoma. Our results confirm that the aglycones Dx-one, 7-deoxyDx-one and Dx-olone can be reliably detected and fully implemented in a common metabolic model. Further it is suggested that pharmacokinetic parameters are similar for low-dose Dx and higher-dosed Dx. There is an increasing body of data suggesting that Dx aglycones might significantly contribute to the (cardiac) toxicity of Dx, and their analysis within pharmacokinetic and pharmacodynamic studies of Dx is therefore suggested to contribute to better understanding and, possibly, better prevention of (cardiac) toxicity.

Acknowledgements M. Joerger is supported by a Novartis-UICC Translational Cancer Research Fellowship funded by Novartis AG and by a research grant from the Swiss National Science Foundation (PBBSB-102331).

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