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NONMEM population pharmacokinetic studies of cytosine arabinoside after high-dose and after loading bolus followed by continuous infusion of the drug in pediatric patients with leukemias

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Abstract We examined the population pharmacokinetics (PPK) of cytosine arabinoside (ara-C) after high-dose ara-C (HDara-C) (3 g/m^2 every 12 h) and after a loading bolus (LB) plus continuous infusion (Cl) of ara-C for 72 h in 52 pediatric patients with leukemias, enrolled in four clinical trials. The PPK analyses of the drug were performed using the NONMEM program. The patients' ages ranged from 2 months to 19 years. The ara-C data were analyzed using a two-compartment open model. Interindividual variability was described by the constant coefficient of variation (CCV) model, while the intraindividual variability was described by a combined additive and CCV error model. The covariates age (AGE) and surface area (SA) were tested to examine their influence on the estimation of the ara-C PPK parameters. In the absence of model covariates, the data fit was characterized by considerable bias, as indicated by the plot of measured vs predicted ara-C concentrations. The fit of the data was greatly improved when the parameters total body clearance (CL), intercompartmental clearance (Q), and volumes of distribution of central (V_{d1}) and peripheral (V_{d2}) compartments were expressed as linear functions of the covariate product, AGE × SA. The final parameter estimates were: $CL = 2.59 \times AGE \times SA$ l/h, $Q = 2.01 \times AGE \times SA$ l/h, $V_{d1} = 0.48 \times AGE \times SA$ l, and $V_{d2} = 38.1 \times AGE \times SA$ l. The coefficients of variation of CL, Q, V_{d1} and V_{d2} were 83.79%, 12.08%, 40.0%, and 52.54%, respectively, indicating substantial interindividual variability. In separate NONMEM analyses, the PK of ara-C and its metabolite uracil arabinoside (ara-U) were modeled simultaneously in order to investigate whether the dependence of ara-C on patient age was due to increased deamination of ara-C to ara-U. The PK of ara-C were described by the two-compartment open model while the PK of ara-U were simultaneously described by the one-compartment open model. The conversion of ara-C to ara-U was modeled as a first-order kinetic process due to the relatively low concentrations of ara-C in plasma. These PPK analyses indicated that elimination of ara-C from the central compartment occurs primarily by its metabolic conversion to ara-U and that the rate of conversion of ara-C to ara-U increases with increasing patient age, which explains the higher ratios of ara-U to ara-C and, hence, the increased ara-C clearance observed in older children as compared to infants. We conclude that the NONMEM PPK methodology allowed the simultaneous analyses of data from different doses and dose regimens and explained phenomena that prior standard two-stage analyses could not.

Key words Nonlinear mixed effects modeling · Pharmacokinetics · Cytosine arabinoside

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Introduction

Cytosine arabinoside (ara-C) is considered to be the most active antimetabolite for inducing remissions in acute myelocytic leukemia (AML) in adults and pediatric patients [1, 2]. In combination with other

anticancer agents, such as anthracyclines, ara-C treatment leads to complete remission (CR) in 60–70% of treated patients [2–9]. Ara-C has been the subject of numerous experimental and clinical studies since its introduction in the early 1960s. During the past 30 years, numerous biochemical and molecular perturbations due to ara-C, as well as pharmacokinetic (PK) and pharmacodynamic parameters of the drug, have been reported [2, 4–13].

Ara-C is not active in the nucleoside form and must be activated to its triphosphate anabolite, ara-CTP, in order to exert its cytotoxic effect [2, 4, 7, 9, 11, 13]. The rate-limiting step in the cascade of activation of ara-C to ara-CTP is on the deoxycytidine kinase (dCk) level, the enzyme which activates the nuleoside to the monophosphate anabolite, ara-CMP [7, 9, 13]. Once ara-CMP has been formed, the triphosphate anabolite (ara-CTP) is thermodynamically favored in mammalian cells both in vivo and in vitro. Thus, the key step in determining the cellular concentrations of ara-CTP lies with the dCk of the target cell. Considerable data are available from adult and pediatric clinical studies regarding optimal concentrations of ara-C in plasma and ara-CTP in leukemic cells [7, 9, 13–16].

Although in neutral aqueous solutions ara-C undergoes deamination to uracil arabinoside (ara-U) in a temperature-dependent manner, ara-C in plasma or whole blood readily undergoes deamination in a reaction that is catalyzed by cytidine deaminase. The most prominent inhibitor of this enzyme is tetrahydrouridine (THU) and to some extent the end-product of the reaction, ara-U [17, 18]. Either one of these inhibitors of cytidine deaminase effectively protects the deamination of ara-C to ara-U in vivo in tumor-bearing mice without additional antitumor benefit [18].

PK studies in pediatric patients with leukemia have shown that ara-C is rapidly eliminated from plasma obeying a two-compartment open model after an intermittent high-dose ara-C (HDara-C) regimen [2, 9, 13]. The average peak ara-C plasma concentrations were approximately 100 μM in one study, and the half-lives of ara-C averaged 17 min and 4 h for the distribution and elimination phases, respectively [13]. The plasma concentrations of ara-U are usually five- to eightfold higher than ara-C, and ara-U is eliminated monoexponentially with an average half-life of 2.3 h [2, 13]. Similar findings have been reported in adult patients [8, 9, 15]. Infant pediatric patients tend to accumulate much higher plasma ara-C concentrations primarily due to the reduced deamination capacity of the body. The average ara-U to ara-C ratio in these pediatric patients is usually 1 to 2, indicating that their capacity to deaminate ara-C is limited or that the clearance is reduced [2, 13, 19]. As a result of this developmental limitation, the plasma concentrations of ara-C in infants are in the range of 300 to 2500 μM [2, 13]; in one case of extreme toxicity a concentration of 5 mM has been achieved [20]. Pediatric patients between 2 and 8 years of age tend to have an ara-U to ara-C ratio of 4 to 5 and the ratio in older children appears to be similar to that determined in adult patients [13–15].

Pediatric patients with higher than 300 μM peak plasma ara-C concentration are most likely to develop CNS toxicity [20]. Conversely, patients with low ($< 100\ \mu M$) peak ara-C plasma concentrations have no manifestations of CNS toxicity. Patients with peak ara-C levels between 101 and 299 μM exhibit a 50% probability of developing neurotoxicity. The significant variability of the PK handling of ara-C by these patients indicates that pharmacological monitoring is necessary after intermittent HDara-C in pediatric patients [20]. Similar observations of high ara-C levels resulting in CNS toxicity have been documented in adult patients [21–23].

There are two types of continuous infusion (CI) administration: simple CI and a loading bolus (LB) followed immediately by CI [8, 14–15, 24]. Using simple CI, the drug achieves steady-state concentrations (C_{ss}) after 6 to 7 half-lives of elimination of the drug, which for some drugs may be a considerable time from a few hours to a few days of the duration of the infusion. An LB followed by CI can achieve instantaneous C_{ss} for any drug with either a short or long half-life of elimination, such as fludarabine, which is maintained by CI [13, 14, 24, 25].

An LB followed by CI of ara-C, either alone or in combination with fludarabine phosphate (F-araAMP), to achieve a specific C_{ss} has been applied successfully in pediatric patients with leukemias [25, 26]. A C_{ss} of ara-C in plasma between 10 and 20 μM is easily tolerable as a 3-day infusion. The determined Km for ara-C in the leukemic cells of a large number of patients has been shown to be relatively low. Thus a C_{ss} between 10 to 20 μM is probably sufficient to achieve optimal activation of ara-C to ara-CTP [13, 14, 18, 27]. One of the major advantages of LB followed by CI of ara-C is that it has the potential to decrease the probability of leukemic cells developing resistance to the drug [28]. This regimen accounts for the deamination of ara-C that takes place in plasma and other tissues. It also provides a constant level of ara-C in plasma and ara-CTP in leukemic cells. Thus there is no time during which leukemic cells could be cycling through S-phase while cellular ara-CTP concentrations are at a nadir between doses [2, 7–9, 13, 15, 29].

Recent population PK (PPK) analyses utilizing the nonlinear mixed effects modeling (NONMEM) program have yielded important information on the pharmacology of many drugs where classical PK analyses have been shown to be inadequate to describe the significant variability among patients. We have used this methodology to retrospectively analyze all of the

pediatric patient ara-C and ara-U PK data [19]. These analyses have been very useful in explaining the variability in ara-C PK between infants and older children [2, 13, 14].

Materials and methods

Drugs

Ara-C was purchased from the Upjohn Co, Kalamazoo, Mich. All other standards for extractions and PK studies were purchased from Sigma Chemical Co., St. Louis, Mo. Other chemicals and solvents for HPLC assays were of analytical or HPLC grade.

Patients

During a period of 12 years, four clinical investigational studies were conducted with HDara-C ($3\,g/m^2$ every $12\,h \times 8$ doses) and LB followed immediately by CI in pediatric patients with leukemias (ALL, AML) and lymphomas ranging in age from 0.17 to 19 years [2, 13, 14]. In the majority of the patients, the duration of HDara-C infusion was 3 h, and in a smaller percentage of children the duration of infusion of the same dose was 1 h [20]. Heparinized blood samples were drawn before the ara-C administration (0 h), and 3, 4, 5, 7 and 9 h after the 3-h administration; for the 1-h ara-C administration, the samples were drawn at 0, 1, 2, 3, 4, 5, 6, 7 and 8 h. For the LB plus the 3-day CI, the samples were drawn at 0, 2, 12, 24, 36, 48, 60 and 72 h. In a few cases, postinfusion specimens were obtained on an hourly schedule. These patients were entered onto one of four separate clinical studies. Ara-C was administered before other anticancer agents such as L-asparaginase, VP-16 (etoposide), 5-azacytidine, or total body irradiation (TBI) in various treatment regimens [2, 13, 14].

HPLC determination of ara-C and ara-U in plasma

Ara-C and ara-U were assayed in intact heparinized plasma on a reverse phase μBondapak C18 column (Waters Associates, Milford, Mass.) by isocratic elution of 5% methanol in 0.05 *M* ammonium acetate, pH 6.5, at a flow rate of 0.8 ml/min. The eluents were monitored by a UV detector at 280 nm and at 0.01 AU scale as described previously [2, 13, 14]. The signal was integrated by a Waters Associates computer program, Waters Expert-840, and concentrations were derived by an external standard method. The HPLC assay for ara-C and ara-U was the same in all studies.

NONMEM PPK analyses

The PPK analyses of ara-C were performed using the computer program NONMEM, version IV, level 2, developed by the NONMEM Project Group of the University of California, San Francisco [30]. This program allows the estimation of average (population) values of PK parameters as well as estimation of inter- and intraindividual variabilities. The clinical data from all four studies (HDara-C and LB + CI) were entered as an appropriate computer file utilized by the NONMEM program.

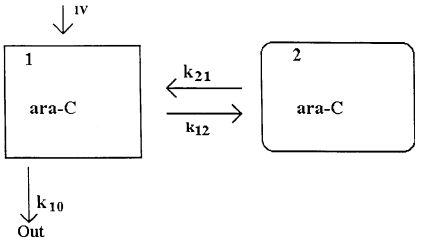

Fig. 1 Schematic representation of the two-compartment open model which describes the PK of ara-C

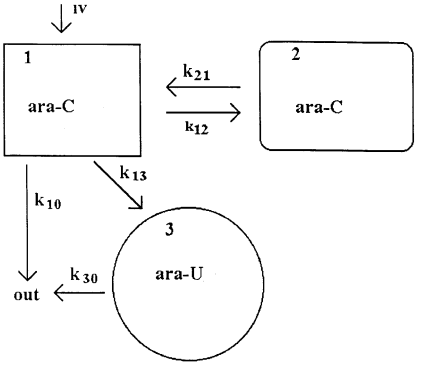

Fig. 2 Schematic representation of the combined PK model which simultaneously describes the PK of the parent drug, ara-C, and its metabolite, ara-U. Ara-C PK data were modeled by the two-compartment open model and ara-U data were modeled by the one-compartment open model

Pharmacokinetic models

Model I

The concentration-time data of ara-C were analysed by a two-compartment model with first-order elimination from the central compartment ultilizing the ADVAN3/TRANS4 subroutines of NONMEM (Fig. 1).

Model II

This model allowed for a simultaneous description of the PK of ara-C and its metabolite ara-U (Fig. 2). The concentration-time data of ara-C were analyzed by a two-compartment open model while those of ara-U were analyzed by a one-compartment open model. The metabolic conversion of ara-C to ara-U was modeled as a first-order kinetic process. Clearance of ara-C from the central compartment (k_{total} rate constant) was described by two processes: (a) deamination of ara-C to ara-U (k_{13}) and (b) renal elimination of parent drug (k_{10}). The differential equations describing the complete model were entered in the ADVAN6 subroutine of NONMEM and they were:

$$dA(1)/dt = -k_{12}A(1) + k_{21}A(2) - k_{10}A(1) - k_{13}A(1) \quad \text{(Eq. 1)}$$

$$dA(2)/dt = k_{12}A(1) - k_{21}A(2) \quad \text{(Eq. 2)}$$

$$dA(3)/dt = k_{13}A(1) - k_{30}A(3) \quad \text{(Eq. 3)}$$

where A(1) is the amount of ara-C in compartment 1, A(2) is the amount of ara-C in compartment 2, and A(3) is the amount of the metabolite ara-U in compartment 3. The microconstants, k_{12}, k_{21}, k_{13}, k_{10}, and k_{30} are the first-order rate constants of transfer or elimination of the drug, ara-C, and its catabolite, ara-U, within the various compartments of the model.

Statistical models

Interindividual variability in PK parameters was expressed as the variance ω^2, of the random error η_i whose mean value is zero, and it was described by the constant coefficient of variation (CCV) model, which is appropriate for skewed parameter distributions [31, 32]. Therefore, an individual's clearance was described by the equation:

$$CL_i = CL(1 + \eta_{CL,i}) \quad \text{(Eq. 4)}$$

where CL_i represents the clearance of ara-C in patient i, CL is the average (population) value of ara-C clearance, and $\eta_{CL,i}$ represents the deviation of this individual's ara-C clearance from the average population value of this parameter. Intraindividual variability of ara-C in models I and II was expressed as the variance σ^2, of random effects ε_{ij}, and was described by a combined additive and CCV error model:

$$C_{ij} = f + f\varepsilon_{1,ij} + \varepsilon_{2,ij} \quad \text{(Eq. 5)}$$

where C_{ij} is the ara-C plasma concentration of patient i at time j, f is the structural PK model, $\varepsilon_{1,ij}$ is the residual error for the CCV portion of the model with variance σ_1^2, and $\varepsilon_{2,ij}$ is the residual error for the additive portion of the model with variance σ_2^2. The two variance terms (additive + proportional) describing intraindividual variability of the ara-C data were estimated in the NONMEM analyses of model I. In model II, these two variance terms were fixed to the values obtained by the NONMEM analyses of model I. This was perceived as necessary in order to reduce estimation time which is considerably large when PK models are expressed as differential equations and when the concentrations of two drugs are modeled simultaneously. Intraindividual variability of ara-U data was described by the CCV model.

Demographic variables (covariates) such as the patients' age (AGE) and surface area (SA) were examined with the PK parameters in order to investigate whether these variables could account for the observed substantial interindividual variability. Improvement of the fit of the data following incorporation of covariates in the regression model was determined based on the minimum value of the objective function (Likelihood Ratio Test), the magnitude of the standard errors of the parameter estimates, the plots of weighted residuals against the predicted concentrations, and the decreases in the estimates of interindividual variabilities. A covariate improved the fit of the data if the log-likelihood difference was statistically significant at the $P < 0.01$ level. The choice of significance level is compatible with previous related works [31, 32], in which it has been argued that small applications of the Likelihood Ratio Test should employ conservative significance levels.

Results

NONMEM pharmacokinetic analyses of ara-C (model I)

The ara-C data obtained after HDara-C and LB + CI were analyzed by a step-wise regression model using the two-compartment open model with first-order elimination from the central compartment. Initially the data were analyzed using a simple regression model (basic model) in the absence of covariates. The variance-covariance (OMEGA) matrix of the basic model was constrained to be diagonal and it calculated interindividual variances for all four basic model PK parameters, i.e. total body clearance (CL), apparent volumes of distribution of the central (V_{d1}) and peripheral (V_{d2}) compartments, and intercompartmental clearance (Q). The plot of predicted (PRED) vs observed (DV) ara-C plasma concentration values indicated significant bias in the fit of the data by this basic analysis, suggesting the need to include covariates in the regression model that could account for the large interindividual variability of the ara-C data (Appendix 1).

The regression model was subsequently expanded to evaluate the effects of the covariates age (AGE) and surface area (SA) on the PK of ara-C. The best model, which resulted in a significant improvement of the data fit (Appendix 2, PRED vs DV) and a substantial decrease in the minimum value of the objective function was the one in which all four basic PK parameters were expressed as linear functions of the covariate product AGE × SA (Appendix 2). The final parameter estimates were: $CL = 2.59 \times AGE \times SA$ l/h, $Q = 2.01 \times AGE \times SA$ l/h, $V_{d1} = 0.48 \times AGE \times SA$ l, and $V_{d2} = 38.1 \times AGE \times SA$ l. The coefficients of variation for CL, Q, V_{d1}, and V_{d2} were 83.79%, 12.08%, 40.0%, and 52.54%, respectively. The results from these analyses are summarized in Table 1.

Table 1 PPK analyses of ara-C data alone (model I). Numbers in parentheses are the standard errors (SE) of the estimated parameters. (*OBF* minimum value of the objective function, *P* covariate product AGE × SA)

Model	CL (l/h)	Q (l/h)	V_{d1} (l)	V_{d2}	ω^2_{CL}	ω^2_0	ω^2_{Vd1}	ω^2_{Vd2}	σ_1^2	σ_2^2	OBF
I–1	15.1 (5.87)	28.7 (13.5)	3.82 (1.94)	77.0 (31.0)	26.9 (27.0)	58.5 (61.1)	1.1×10^3 (9.5×10^2)	0.29 (1.42)	0.005 (0.017)	613.0 (237.0)	3410.49
I–2	$2.60 \times P$ (0.62)	$1.82 \times P$ (0.86)	$0.46 \times P$ (0.22)	$34.6 \times P$ (22.6)	0.65 (0.24)	0.17 (0.30)	0.017 (0.32)	0.43 (0.70)	0.13 (0.07)	122.0 (39.9)	2837.97

Table 2 Simultaneous PPK analyses of ara-C and ara-U data (model II). Numbers in parentheses represent the standard errors of the estimated parameters. The variance terms σ_1^2 and σ_2^2 of the ara-C data were assigned fixed values. The variance term σ_3^2 of the ara-U data was estimated during the NONMEM analyses (*OBF* minimum value of the objective function)

Model	k_{12} (h^{-1})	k_{21} (h^{-1})	k_{10} (h^{-1})	k_{13} (h^{-1})	k_{30} (h^{-1})	V_{d1} (l)	V_{d3} (l)	σ_1^2	σ_2^2	σ_3^2	OBF
II-1	4.89	0.24	3.0×10^{-9}	3.65	3.78	1.50	0.47	0.13	107.0	0.06	7456.82
II-2	4.89	0.24	–	3.65	3.78	1.50	0.47	0.13	107.0	0.06	7456.82
II-3	1.16 (0.30)	0.08 (0.01)	–	$1.53 \times$ AGE (0.25)	0.41[b] (0.06)	1.37 (0.51)	4.98 (1.50)	0.13	107.0	0.10 (0.40)	6764.14
II-4[a]	1.37 (0.48)	0.07 (0.04)	$1.19 \times$ AGE (0.13)	–	–	2.17 (0.39)	– –	0.38 (0.19)	67.5 (29.7)	–	2810.17

[a] This model estimated the PK parameters of ara-C along using the ADVAN3/TRANS1 subroutines of NONMEM
[b] The half-lives of elimination ($t_{1/2,el}$) of ara-U were 0.18 h and 1.69 h for models II-1, II-2, and II-3, respectively. The estimates of $t_{1/2,el}$ in models II-1 and II-2 were highly biased while $t_{1/2,el}$ of ara-U in model II-3 was very similar to that estimated by STS analyses of the drug [2, 13]

NONMEM pharmacokinetic analyses, of ara-C/ara-U data (model II)

The ara-C and ara-U data were analyzed simultaneously using model II. The ara-C data obtained after HDara-C and LB + Cl were described by the two-compartment open model while the ara-U data were described by the one-compartment open model, as previously determined by the standard two-stage (STS) analyses of these compounds [2, 13, 14]. Initially, the ara-C and ara-U data were analyzed by a simple regression (basic) model that did not include covariates. The variance-covariance (OMEGA) matrix was constrained to be diagonal. Intraindividual variability of ara-C was described by a combined additive and proportional (CCV) error model, in which the two variance terms, σ_1^2 and σ_2^2, were fixed to the final estimates obtained in the NONMEM analyses of ara-C using model two, Table 1 (I–2). Intraindividual variability of ara-U was described by the CCV model. Elimination of ara-C from the central compartment was described by two first-order rate constants: k_{13} which describes the metabolic conversion of ara-C to ara-U and k_{10} which describes renal elimination of ara-C. The NONMEM analysis of ara-C and ara-U data using this model estimated a k_{10} approximately equal to zero and a $k_{13} = 3.65\ h^{-1}$ ($t_{1/2,el} = 0.19$ h). Therefore, the model was modified so that clearance of ara-C from the central compartment was described only by k_{13}, i.e. clearance of ara-C from the central compartment occurred via its metabolic conversion to ara-U, rather than by renal elimination of parent drug.

The observed differences between the ratios of ara-U to ara-C in infants and older children suggested the possibility of an age-dependent conversion of ara-C to ara-U. The ratio of ara-U to ara-C was approximately 1 in infants and averaged 7 in older patients [2]. To examine the effect of the covariate AGE on the ara-U/ara-C ratio, the parameter k_{13} was expressed as a linear function of AGE as shown in Eqs. 6 and 7 below:

$$k_{13} = \theta(1) + \theta(2) \times \text{AGE} \quad \text{(Eq. 6)}$$

$$k_{13} = \theta(2) \times \text{AGE} \quad \text{(Eq. 7)}$$

where $\theta(1)$ and $\theta(2)$ are parameters which express k_{13} and are estimated by NONMEM. Expression of k_{13} in terms of AGE in Eq. 6 was highly significant as determined by the Likelihood Ratio Test. However, the estimate of $\theta(1)$ in Eq. 6 was approximately zero, suggesting that this parameter was not important in the model and a more simplified expression for k_{13} should be used. This was accomplished by utilizing Eq. 7. Table 2 shows the parameter estimates obtained for the basic and expanded regression models. The PK parameters of ara-C, k_{12}, k_{21}, k_{13}, and V_{d1} estimated using model II-3 (Table 2), are very similar to the same PK parameters of ara-C estimated when the ara-C data were analyzed alone (Table 2, model II-4), by the two-compartment open model with first-order elimination from the central compartment (ADVAN3/TRANS1 subroutines of NONMEM). The final parameter estimates of both ara-C and ara-U for model II-3 were: $k_{12} = 1.16 \pm 030\ h^{-1}$, $k_{21} = 0.08 \pm 0.01\ h^{-1}$, $k_{13} = 1.53 \times \text{AGE}\ h^{-1}$, $V_{d1} = 1.37 \pm 0.51$ l, $k_{30} = 0.41 \pm 0.06\ h^{-1}$ ($t_{1/2,el} = 1.69$ h), and $V_{d3} = 4.98 \pm 1.50$ l.

Discussion

The PK of ara-C and its catabolite ara-U have been evaluated by STS methodology in four separate clinical

studies in pediatric patients [2, 13, 14]. The STS analyses of the ara-C PK data did not explain the significant variabilities in elimination and peak plasma levels in younger children. We have utilized NONMEM PK modeling to examine the same PK data of ara-C and ara-U from 52 pediatric patients, with leukemias or lymphomas receiving HDara-C or LB followed by CI ara-C, in order to explain some of these pharmacological variabilities [19]. The HDara-C data had shown significant intra- and interindividual variability in the peak plasma ara-C concentrations [2, 13]. In order to reduce PK variability, and based on preliminary PK data, the LB followed immediately by Cl regimen was designed and applied successfully to pediatric patients with leukemias [13, 14, 24]. The recommended doses of the LB + CI ara-C regimen, for each of the dose level escalations have been shown to reproduce the plasma concentrations of ara-C in subsequent studies [14, 26]. However, there was a greater than a 100-fold difference in the peak plasma concentration of ara-C after the HDara-C regimen and approximately a 10-fold difference in the half-life of elimination of the drug in these pediatric patients. Since the high ara-C plasma concentrations are associated or correlated with other organ and CNS cerebellar toxicities in patients, we utilized population modeling to better understand the PK variabilities of the drug and the possible PK factor(s) associated with these toxicities [8, 13, 14, 20, 27, 33–36]. These high plasma ara-C concentrations were observed in the infant patients who were receiving HDara-C regimen [2, 20, 24, 33].

Among the different PK methods used to estimate ara-C CL and V_d in pediatric patients are the STS method and NONMEM. The first method was applied in the original analysis of the phase I clinical studies conducted in these patients [2, 13, 14, 27]. Since then the NONMEM method had been developed and was used to retrospectively analyze the data from the HDara-C and the LB + CI regimens and the results are shown in this report [19].

The ara-C data were analyzed by NONMEM methodology using the two-compartment open model, which was the model previously determined by the STS method to describe the PK of this drug [2, 13, 14]. Our NONMEM analyses indicated considerable bias in the fit of the data (Appendix 1) and the estimated ara-C PK parameters (Table 1, model I). Examination of the effects of covariates on the PK of ara-C indicated that substantial interindividual variability could be attributed to differences of age and size (surface area) among the patients. The total body clearance, apparent volumes of distribution of the central and peripheral compartments, and intercompartmental clearance of ara-C had statistically significant linear relationships with the covariate product AGE × SA, with their values increasing with increasing patient age (AGE) and surface area (SA). The results from these analyses could, therefore, explain the lower ara-C plasma concentrations in older children as compared to infant and younger patients. In an effort to determine whether the increasing values of ara-C clearance with increasing AGE were due to differences in the capacity of different age groups to deaminate ara-C to ara-U, as the experimentally determined ara-U/ara-C ratios suggested, the PK of ara-C and ara-U were modeled simultaneously (model II) as a limited physiological model. These analyses indicated that clearance of ara-C from the central compartment occurred primarily via its deamination to ara-U and that the rate of ara-C deamination to ara-U increased with increasing age as the expected enzymatic capacity of the body increased. Hence, patient age was significant in explaining interindividual variability of ara-C plasma levels, strongly suggesting that infant patients, who have an underdeveloped metabolic system, do not eliminate plasma ara-C, via its conversion to ara-U, as effectively as older children, leading to the higher plasma concentrations of administered drug. This finding confirms the previous determination that the infant patients were accumulating very high ara-C concentrations and had a long half-life of elimination of ara-C, a subset condition that STS PK analyses could not explain.

In conclusion, the NONMEM PPK analyses allowed us to simultaneously analyze data from different doses and dose regimens (HDara-C ad LB + CI) and explain the large variability of the ara-C concentration data among the different patients. In addition, using the NONMEM program we were able to simultaneously model the PK of the parent drug, ara-C, and its metabolite, ara-U, and thus determine that the higher ara-C plasma concentrations in infants and younger children could be attributed to the lower capacity of this subset of patients to efficiently deaminate ara-C to ara-U. In future studies we plan to enrich this NONMEM model by including additional patient data of ara-C and ara-U, as well as data of the intracellular anabolite ara-CTP in leukemia patients, in order to better understand the pharmacology of this important antileukemic drug. The cellular ara-CTP data could be modeled in a similar manner using Mcihaelis-Menten kinetic parameters, for which information is readily available for pediatric patients with leukemias.

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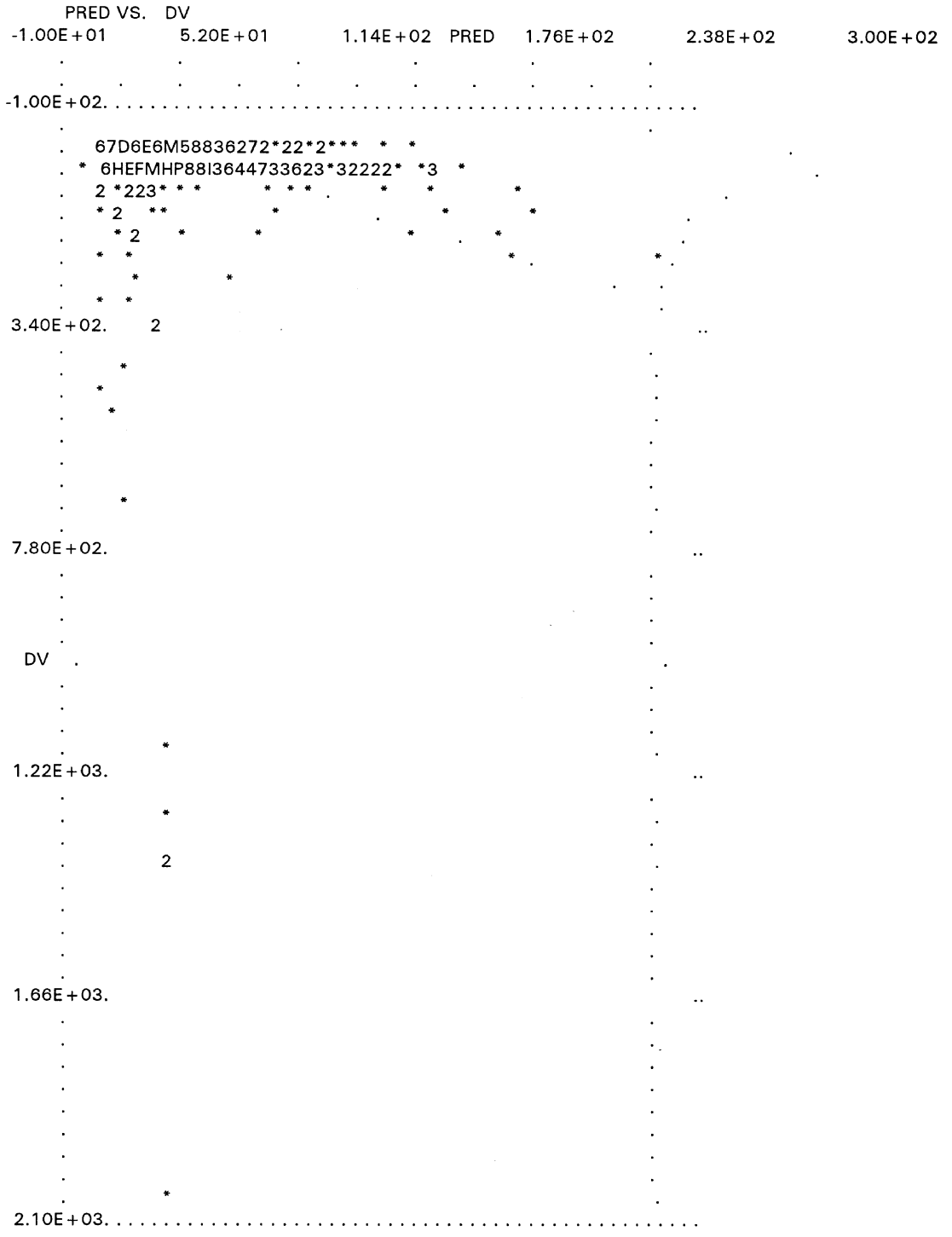
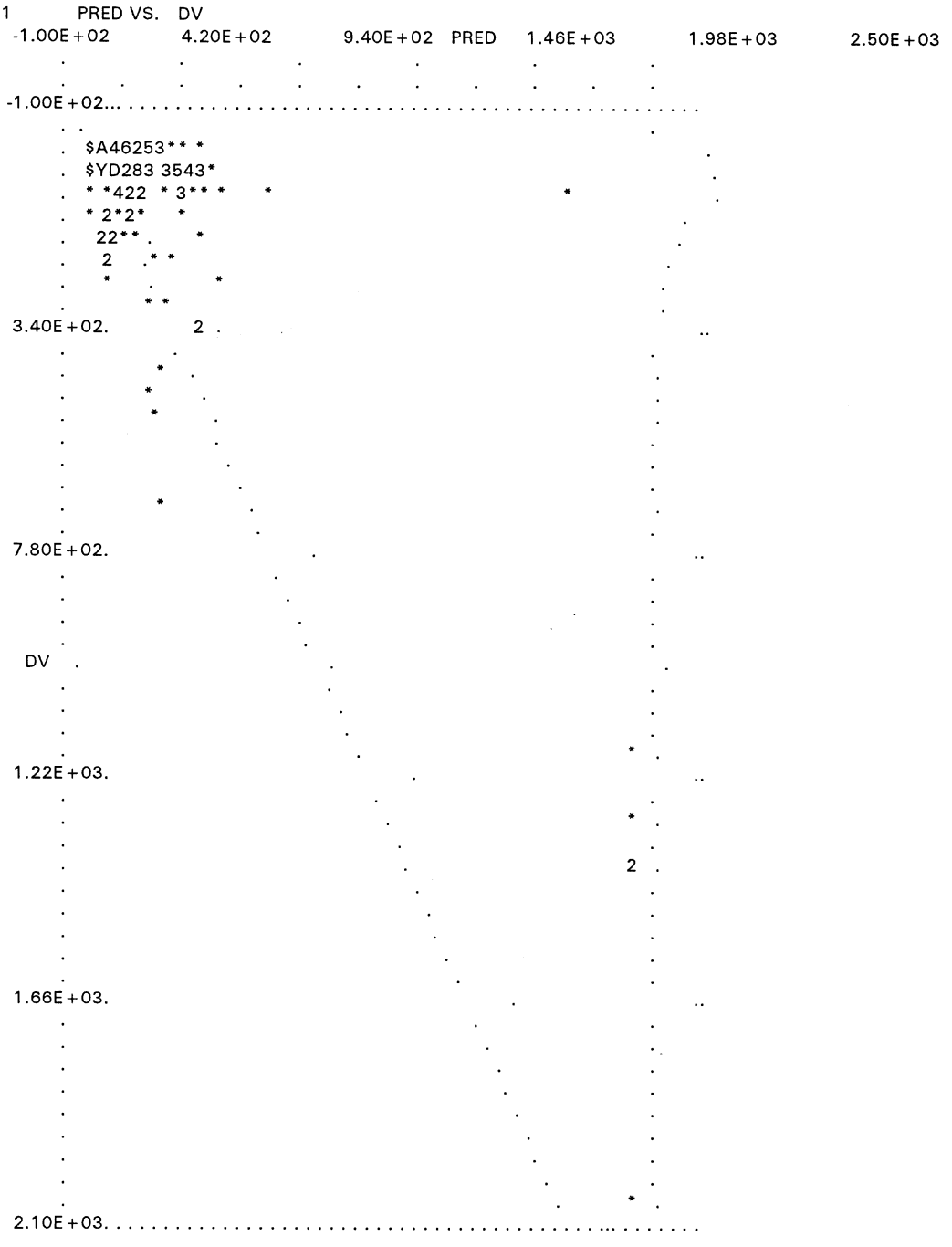
Appendix

Appendix 1



Appendix 1 Plot of predicted (PRED) vs observed (DV) ara-C plasma concentration values using basic model I-1. This plot indicated significant bias in the fit of the data by this basic model analysis

Appendix 2



Appendix 2 Plot of predicted (PRED) vs observed (DV) ara-C plasma concentration values using an expanded regression model (model I–2). Model I–2 resulted in a significant improvement of the data fit suggesting that the covariates AGE and SA could explain a large portion of interindividual variability

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