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Non-linear mixed effects modeling of sparse concentration data from rats: Application to a glycogen phosphorylase inhibitor

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SUMMARY

We investigated the use of non-linear mixed effects modeling in two preclinical studies of the glycogen phosphorylase inhibitor 1,4-dideoxy-1,4-imino-D-arabinitol (DAB). In a 28-day repeated-dose toxicity study rats were dosed once daily p.o. with 0, 20, 45, 100, or 470 mg/kg of DAB in aqueous solutions by oral gavage. Three blood samples were obtained from each animal using a staggered sampling scheme. During the cause of model development, data were included from a safety pharmacological cardiovascular study, in which rats were dosed once orally with 0, 4, 40, or 400 mg/kg of DAB thereby enabling an extension of the dose range of the model. DAB was assayed in plasma using a validated LC/MS/MS method. Non-linear mixed effects modeling was performed using the software NONMEM. The covariate analysis comprised dose, sex and time. Exposure results (C_{max}, AUC) obtained by mixed effects modeling were compared to results from noncompartmental analysis using naïve pooling of data. The final model was a one-compartment model with first order absorption and a saturation-like dose dependent increase of the (oral) clearance (CL/f) and volume of distribution (V/f). Furthermore, V/f increased (by 55%) from Day 1 to Day 28. The dose dependencies of CL/f and V/f were most likely due to dose dependent decreases of the fraction systemically absorbed (f). The mechanism behind the dose dependencies may be saturation of a (putative) carrier mediated transport or modulation of tight junctions causing a reduced paracellular transport across the intestinal epithelium. Exposure results obtained from the model compared well with results obtained using noncompartmental analysis. An analysis of the data requirements for non-linear mixed effects modeling showed that at least three concentration values per animal were required for model development. We conclude that non-linear mixed effects modeling is feasible even with dose dependent pharmacokinetics in preclinical studies, such as 28-day toxicity studies in rodents. Supplementing data from additional preclinical studies may be required in order to extend the dose range. Non-linear mixed effects models may prove to be valuable tools in early PK and PK-PD modeling during drug development.

INTRODUCTION

The main objective of toxicokinetic studies is to assess drug exposure and thereby assist in the safety assessment of new investigative drugs. This has traditionally been accomplished by means of noncompartmental analysis (NCA) for estimation of relevant pharmacokinetic (PK) parameter values such as the peak concentration (C_{max}), the time to reach peak concentration (t_{max}) and the area under the concentration-time curve (AUC) in plasma (1). In non-rodents, full plasma concentration profiles allow for the estimation of individual PK parameter values. Rodents on the other hand are typically sampled once or

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twice per time sampling day and thus, NCA is performed using data files of pooled (or averaged) concentration-time values from all animals at each time and in each dosing group as if these values originate from the same animal (naïve pooling).

Whereas NCA is an efficient technique for quantifying drug exposure, it is less useful for simulating the outcome of future PK-studies and for describing the pharmacokinetic-pharmacodynamic (PK-PD) relationship. Non-linear mixed effects modeling offers an opportunity for achieving these goals (2). A population pharmacokinetic (PopKin) model consists of a compartmental PK model (the structural model) as well as intra- and inter-individual variance models (error models). For each PK parameter of the structural model, a population mean and variance are estimated and in addition, individual estimates of PK parameter values are obtained by means of empirical Bayesian estimation (3). An important feature of the population approach is the opportunity to explain part of the inter-individual pharmacokinetic variability by means of covariates, such as demographic and environmental parameters.

PopKin modeling is particularly advantageous in studies with sparse blood sampling but may also be applied to full concentration-time profiles. It is gaining widespread use in clinical studies, in particular in Phase III studies. However, due to the ability to handle sparse sampling, PopKin modeling seems to be an ideal tool for toxicokinetic studies, and a few studies have presented such models (2, 4-7). Furthermore Cosson et al., 1997 have shown that pharmacokinetic models for different species may be combined in a single population model thereby enabling allometric interspecies scaling of clearance and volume of distribution (8).

The purpose of this study was to investigate the use of a population approach for the toxicokinetics from a 28-day toxicity study in rats of the glycogen phosphorylase inhibitor DAB [1,4-dideoxy-1,4-imino-D-arabinitol]. The results were compared with those obtained using traditional NCA. In order to expand the dose range of the model, the feasibility of combining data from the toxicity study with a cardiovascular study was investigated. Furthermore, the data file used for PopKin modeling was reduced from three to two samples per animal in order to investigate the data requirements using this technique.

MATERIALS AND METHODS

Animal study design

Data were obtained from two preclinical studies in the rat. Doses in the text refer to free DAB base.

The first study was a repeated-dose oral toxicity study of 28-day duration in Sprague-Dawley rats. Ten rats of each sex randomly allocated to five groups were dosed once daily by oral gavage for 28 days with vehicle, 20, 45, 100, or 470 mg/kg of DAB in aqueous solutions. Blood samples for toxicokinetic analysis were collected during superficial CO_2 / O_2 anesthesia from the ophthalmic venous plexus on the first and last day of dosing (Day 1 and 28).

For each sex and dose level on Day 1, two blood samples per rat were collected; on Day 28, one sample per rat was collected. The sampling scheme (Table I) enabled collection of 10 samples till 10 hours after drug administration with a different sampling scheme for each rat.

Table I: Blood sampling scheme used in the 28-day toxicity study.

Rat No.	Blood sampling post dosing [h]									
	Predose	0.17	0.33	0.67	1	1.5	2	5	8	10
1	A									B
2		A							B	
3			A					B		
4				A			B			
5		B			A					
6			B			A				
7						B	A			
8					B			A		
9				B					A	
10	B									A

Day 1: A & B collected
Day 28: B collected

The second study was a safety pharmacology study investigating the effects of DAB on cardiovascular parameters in conscious telemetry monitored Sprague-Dawley rats. Two female rats from each dosing group were dosed once orally by gavage with 4, 40 or 400 mg/kg of DAB. Twelve blood samples were collected till 10 hours after dosing with two samples per rat and one sample per time using a staggered sampling scheme similar to the one chosen in the previous study. Data from the safety pharmacology study were too sparse to allow for traditional pharmacokinetic interpretation, but valuable for expanding the population pharmacokinetic model built for the toxicity study to encompass doses lower than 20 mg/kg/day. Both studies were performed in compliance with international regulations (OECD-GLP guidelines).

Pharmacokinetic evaluation

Non-compartmental approach (NCA)

For each combination of dose level, sex and sampling occasion (i.e. 4 dose levels, 2 sexes, 2 sampling occasions), the data from 10 animals were combined into a profile using naïve data pooling. On Day 1 the mean of two concentrations at each time was used for NCA. On Day 28 the profile consisted of only one concentration per time. The maximum plasma concentration (C_{max}) and the corresponding time (t_{max}) were determined by inspection of the naïve concentration-time profile. The total area under the plasma concentration vs. time curve (AUC) on Day 1 was determined by the linear trapezoidal rule using conventional extrapolation technique to infinity. On Day 28, the area under the plasma concentration-time curve within a dosing interval ($AUC^{ss}_{0\text{-}24h}$) was calculated using the trapezoidal rule. NCA was performed using WinNonlin V1.1 (Pharsight, Mountain View, CA, USA).

Population pharmacokinetic analysis

Plasma concentrations below the lower limit of quantification (LLOQ) including predose samples Day 1 were omitted from the analysis. The data file from the toxicity study contained 195 concentration values from 75 rats. In total 50 plasma concentrations were below the LLOQ.

Non-linear mixed effects modeling and estimation by extended least squares regression was performed using the software NONMEM version V, level 1.1 (University of California San Francisco, USA), installed on an IBM 300 PL microcomputer. NONMEM was run under Visual-NM version V (R.D.P.P., Montpellier, France), a Windows based interface to NONMEM containing graphical and statistical tools. The structural model was a one-compartment open model with first order absorption and elimination (PREDPP subroutines ADVAN2/TRANS2). The first-order (FO) method was used for estimation.

The pharmacokinetic parameters in the model were the oral clearance (CL/f), the apparent volume of distribution (V/f) and the absorption rate constant (k_a), f being the fraction of the dose systemically available. The interindividual variability of these parameters was modeled using exponential error models:

$$CL/f = TVCL \cdot \exp(\eta_1)$$
$$V/f = TVV \cdot \exp(\eta_2)$$
$$k_a = TVKA \cdot \exp(\eta_3)$$

where TVCL, TVV and TVKA are the population means (typical values) of CL/f, V/f and k_a, respectively. η_1, η_2 and η_3 are normal distributed random variables with means equal to zero and variances ω_{CL}^2, ω_V^2, and ω_k^2, respectively. The intraindividual variability of the plasma concentrations $C_i(t)$ was also modeled using an exponential error model:

$$C_i(t) = C_i^*(t) \cdot \exp(\varepsilon_i(t))$$

where $C_i^*(t)$ is the expected plasma concentration for the i´th individual for a particular dose and time and $\varepsilon_i(t)$ is a normal distributed variable with zero mean and variance σ^2. The parameters estimated in the basic model without covariates were TVCL, TVV, TVKA, ω^2_{CL}, ω^2_V, ω^2_{ka}, and σ^2.

Covariate relationships were identified using NONMEM by forward inclusion and backwards elimination of the three possible covariates: dose, sex and sampling occasion. Each covariate was during forward inclusion separately added to the model and typical values, interindividual and residual variability, objective function values (OBJF), plots of observed versus predicted plasma concentrations and of weighted residuals versus predicted concentrations were examined. Covariates that emerged as significant ($P < 0.05$, α: 3.84 for 1 df) by the likelihood ratio test were incorporated into the pharmacokinetic model. Plots of empirical Bayes estimates helped to define covariate relationships. Next, all covariate relationships identified during forward inclusion were added into one model to give the full model. During backward elimination, each covariate added during forward inclusion was sequentially removed from the full model and evaluated by the likelihood ratio test. The criteria used at this stage for retaining a covariate was an increase in DOBJF at removal of the covariate of at least 10.8 corresponding to $P<0.001$ in a chi-square distribution with 1 df (for covariate relationships with one additional parameter). The resulting model was adopted as the final model.

Based on the final population pharmacokinetic model, the AUC-values were computed as f·Dose/CL and k as CL/V. The C_{max} following the first administration (Day 1) was calculated as:

$$C_{max} = \frac{Dose \cdot f}{V} \cdot e^{-k \cdot t_{max}} \text{ where } t_{max} = \frac{\ln\left(\frac{k_a}{k}\right)}{(k_a - k)}$$

The equation used for calculating maximum concentration at steady-state (C^{ss}_{max}) was:

$$C^{ss}_{max} = \frac{Dose \cdot f \cdot k_a}{V(k_a - k)} \left(\frac{e^{-k \cdot t^{ss}_{max}}}{1 - e^{-k \cdot \tau}} - \frac{e^{-k_a \cdot t^{ss}_{max}}}{1 - e^{-k_a \cdot \tau}} \right) \text{ where}$$

$$t^{ss}_{max} = \frac{\ln\left(\frac{k_a \cdot (1 - e^{-k \cdot \tau})}{k \cdot (1 - e^{-k_a \cdot \tau})}\right)}{(k_a - k)}$$

Initially, a population pharmacokinetic model was created using all available concentration data from the toxicity study. However, we also examined what impact a reduction of the data file would have on the selection of the final population pharmacokinetic model. For this purpose the sparse sampling scheme of 2 and 1 sample(s) per time Day 1 and 28, respectively was reduced to 1 sample per animal per sampling occasion.

Finally, AUC and C_{max} values calculated by the NCA approach were compared to the corresponding parameters from the final NONMEM model.

Drug assay

DAB was assayed in plasma by LC/MS/MS following protein precipitation. A deuterium labeled analogue of DAB was used as internal standard (IS). Ten µl of plasma were pipetted to tubes placed on water-ice, 500 µl solution holding 50 ng/ml of IS in methanol (analytical grade, Merck, Darmstadt, Germany) was added and the tubes centrifuged at 2520 g for 5 minutes at 4°C. The supernatant (300 µl) was transferred to clean tubes and evaporated to dryness at 50°C using a TurboVap LV evaporator (Zymark, Hopkinton, MA, USA). The residues were reconstituted in 150 µl of methanol-2.5 mM ammonium acetate buffer pH 6.0 (70:30, v/v) and centrifuged at 2520 g for 10 minutes at 4°C. One hundred µl were transferred to a 96-well microtiter plate and 25 µl injected into the LC/MS/MS system. A Perkin Elmer (PE) Sciex API 3000 triple-quadropole mass spectrometer (Sciex, Thornhill, Canada) equipped with a TurboIonSpray interface was used as a detector in the chromatographic system. The system was operated with an ionspray and orifice voltage of 5000 V and 30 V, respectively; the interface temperature was set at 250°C using a nitrogen nebulizing gas flow of 10 l/min. The liquid chromatographic system consisted of a PE Series 200 LC pump (Perkin Elmer, Norwalk, CT, USA) and a Gilson 233 XL autosampler (Gilson, Villier Le Bel, France). Isocratic elution was accomplished using a LiChrosorb diol column (50 mm x 3 mm, 5 µm) from Merck, Darmstadt, Germany and a mobile phase consisting of methanol-2.5 mM ammonium acetate buffer pH 6.0 (70:30, v/v) at a flow rate of 0.6 ml/min. The flow was split and 100 µl/min was led into the interface. The run time was 4 minutes and retention times were 2:30 min for both DAB and IS. Multiple Reaction Monitoring (MRM) was performed monitoring precursor ions m/z 134.0 (DAB) and 136.0 (IS), and product ions m/z 98.0 (DAB) and m/z 100.0 (IS). Data processing and quantification was performed using the PE Sciex MacQuan version 1.6. The assay was validated and found linear in the concentration range of 150-24000 ng/ml. At all concentration levels, within and total assay precision (CV %) was < 13.7%; within assay accuracy varied between 90-107%. The LLOQ and ULOQ (lower and upper limit of quantification) were found to be 150 and 24000 ng/ml, respectively and the recovery of both analyte and IS was 95%. Interfering peaks from endogenous substances were not present in the chromatograms at the retention times of DAB and IS.

RESULTS

Model building

Two models of the pharmacokinetics of DAB in rats were developed. The first model was based on data from the toxicity study only and next, a model was fitted using pooled data from the toxicity study and a cardiovascular study in rats. One-compartment models with first order absorption and elimination were fitted throughout.

The covariate analysis of the toxicity study revealed significant effects of dose on both CL/f and V/f as well as a change of V/f with time (Table II).

The dose dependencies at this stage were modeled as:

$$\frac{Cl}{f} = \frac{\theta_1 \cdot Dose}{\theta_2 + Dose} \text{ (Eq. 1) and } \frac{V}{f} = \frac{\theta_3 \cdot Dose}{\theta_4 + Dose}, \text{ (Eq. 2)}$$

the θ's being constants. As seen from Table II, a non-linear dose dependency was most adequate for CL/f (Model No. 3 vs. 2). Conversely, for V/f, a linear dose dependency provided the best fit during initial forward inclusion of covariates (Model No. 4 vs. 5), but later during model refining, the non-linear model proved to be superior (Model No. 17 vs. 18). The reason for this discrepancy is not clear.

Table II: Overview of the covariate analysis of complete versus reduced data files of the 28-day toxicity study.

	Model No.	Complete data file: Model	ΔOBJF	Significance	Reduced data file A: ΔOBJF	Significance	Reduced data file B: ΔOBJF	Significance
Forward inclusion of covariates	1	Basic model[1]	0.0	-	0.0	-	0.0	-
	2	Dose dependent CL/f (linear)	-3.2	ns	-2.2	ns	-4.2	p<0.05
	3	Dose dependent CL/f (nonlinear)	-12.1	p<0.001	-7.0	p<0.01	-9.1[5]	p<0.005
	4	Dose dependent V/f (linear)	-127.0	p<0.001	-63.3	p<0.001	-82.4	p<0.001
	5	Dose dependent V/f (nonlinear)	-106.2	p<0.001	0.0	ns	-123.5	p<0.001
	6	Sex dependent CL/f	-4.1	p<0.05	-1.4	ns	5.2[5]	p<0.05
	7	Sex dependent V/f	-4.5	p<0.05	-6.7	p<0.01	-4.2	p<0.05
	8	Time dependent CL/f	-8.5	p<0.005	-0.3	ns	-18.2	p<0.001
	9	Time dependent V/f	-29.1	p<0.001	-15.2	p<0.001	-21.1	p<0.001
Backwards elimination of covariates	10	Full model[2]	0.0	-	0.0	-	0.0	-
	11	Full model + dose dependent CL/f (nonlinear)	45.0	p<0.001	18.8	p<0.001	54.6	p<0.001
	12	Full model + dose dependent V/f (linear)	141.7	p<0.001	101.5[5]	p<0.001	117.9[5]	p<0.001
	13	Full model + sex dependent CL/f	0.4	ns	2.0	ns	0.1	ns
	14	Full model + sex dependent V/f	2.1	ns	10.9[5]	p<0.001	4.0	p<0.05
	15	Full model + time dependent CL/f	0.9	ns	14.5	p<0.001	2.7	ns
	16	Full model + time dependent V/f	29.4	p<0.001	28.6	p<0.001	22.9	p<0.001
Resulting model	17	Dose dependent CL/f (nonlinear) Dose dependent V/f (linear) Time dependent V/f	4.2[3]	ns	18.7[3]	p<0.001	11.7[3,5]	p<0.001
Refined model	18	Dose dependent CL/f (nonlinear) Dose dependent V/f (nonlinear) Time dependent V/f	-17.8[4]	Final model	-16.2[4]	p<0.001	-18.1[4,5]	p<0.001

[1] Model without covariates
[2] Model with dose dependent CL/f (non linear) and V/f (linear), sex dependent CL/f and V/f, and time dependent CL/f and V/f.
[3] Compared to the full model (model 10).
[4] Compared to model 17.
[5] A fixed value had to be used for k_a in order to obtain convergence (k_a=10). ns: not significant

The time dependency of V/f was modeled as V/f (Day 28) = θ_5 ·V/f (Day 1) as only two time points were represented in the data file. According to this model, the population value of V/f increased by 55% from Day 1 to Day 28 of the study (Table III).

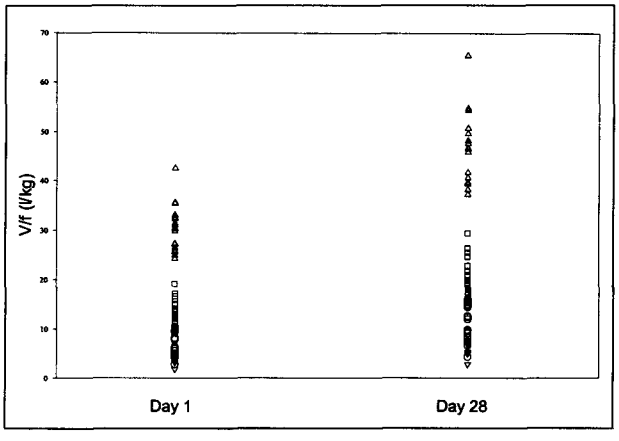

Fig. 1 : Individual predicted values of V/f on Day 1 and Day 28 from the final model of the 28-day toxicity study in rats. The dose levels were: 20 (V), 45 (O), 100 (□) and 470 (Δ) mg/kg.

The individual Bayes estimates of V/f on Day 1 and Day 28 are shown in Figure 1.

The initial models of the dose dependencies of CL/f and V/f (Eq. 1 and 2) imply that the clearance and volume of distribution values approach zero as the dose approaches zero, which is not realistic. Consequently, after completing the analysis of data from the toxicity study, the data file was supplemented with data from a cardiovascular study of DAB in rats in order to include data from lower dose levels. This combined data file comprised doses in the range of 4-470 mg/kg.

The models for CL/f and V/f were now changed to:

$$\frac{Cl}{f} = \theta_5 + \frac{\theta_1 \cdot Dose}{\theta_2 + Dose} \quad \text{(Eq. 3) and}$$

$$\frac{V}{f} = \theta_6 + \frac{\theta_3 \cdot Dose}{\theta_4 + Dose} \quad \text{(Eq. 4),}$$

thus allowing CL/f and V/f to approach non-zero baseline values as the dose approaches zero. Otherwise, the dual-study model was structurally identical to the single-study

Table III: Structural and error parameters for the final models.

Study	Structural (covariate) model	Error model	
		Parameter	Estimate ± S.E.
28-day toxicity study	$k_a = 8.22 \pm 2.09\ (h^{-1})$	ω^2_{ka}	–[a]
	$\frac{CL}{f} = \frac{(7.17\pm1.06)\cdot Dose}{(67.2\pm19.8)+Dose}\ (l\cdot h^{-1}\cdot kg^{-1})$	ω^2_{CL}	.832 ± 0.432
	$\frac{V}{f} = \frac{(47.8\pm9.8)\cdot Dose}{(231\pm67)+Dose}\ (l\cdot kg^{-1})$	ω^2_{V}	.146 ± 0.86
	$\frac{V}{f}(Day\,28) = (1.54\pm0.15)\cdot \frac{V}{f}(Day\,1)$	σ^2	.200 ± 0.43
28-day toxicity study combined with CV effect study	$k_a = 8.71 \pm 2.39\ (h^{-1})$	ω^2_{ka}	–[a]
	$\frac{CL}{f} = (0.785\pm0.407) + \frac{(7.22\pm1.26)\cdot Dose}{(119\pm49)+Dose}\ (l\cdot h^{-1}\cdot kg^{-1})$	ω^2_{CL}	.0992 ± 0.395
	$\frac{V}{f} = (0.276\pm0.471) + \frac{(44.7\pm8.1)\cdot Dose}{(233\pm70)+Dose}\ (l\cdot kg^{-1})$	ω^2_{V}	.127 ± 0.747
	$\frac{V}{f}(Day\,28) = (1.55\pm0.14)\cdot \frac{V}{f}(Day\,1)$	σ^2	.189 ± 0.38

[a] Interindividual variation of k_a could not be estimated
CV: cardiovascular

model. Figure 2 shows the individual Bayes estimates and the model predicted estimates for CL/f and V/f obtained for this model (dose range 4 - 470 mg/kg).

Although the combined data file allowed for the estimation of non-zero values of both CL/f and V/f at doses approaching zero, only CL/f could be estimated with reasonable precision (Table III). Thus, the model predicted value of V/f at zero dose was 0.276 ± 0.471 l/kg and was not significantly different from zero (details not shown). The parameter estimates of the dual-study model were close to those of the single-study model apart from θ_2, which increased from 67.2 to 119 (Table III). Figure 3 shows the observed and model predicted concentration values at each dose level for the dual-study model.

As seen, reasonable fits were obtained at all dose levels although at some dose levels (100 mg/kg Day 28; 45 and 20 mg/day at Day 1) the data showed a tendency towards an additional compartment that was not accounted for by the model.

Comparison with NCA

The results obtained from the single-study population model were compared with those obtained by NCA. Only the key parameters for demonstrating drug exposure, C_{max} and AUC, were compared in this study. As seen in Figure 4, the results obtained using the two methods of calculation were in good accordance. The largest differences were seen for C_{max} on Day 28.

Results obtained using reduced data files

Finally, we investigated whether the data file used for population modeling could be reduced without affecting the outcome of the model building process. For this purpose, the data file from the 28-day toxicity study was reduced by deleting one of the two concentration values on Day 1, thus obtaining a total of 2 samples per animal instead of three values in the original file. The reduction was done using two different approaches, either by eliminating the diagonal time points (Table I, A samples) or by eliminating the off-diagonal time points Day 1 (Table I, B samples).

Using these data files (Reduced files A and B, respectively), the entire model building process was repeated, fitting the same models as with the full data files. In order for some of the models to converge during fitting,

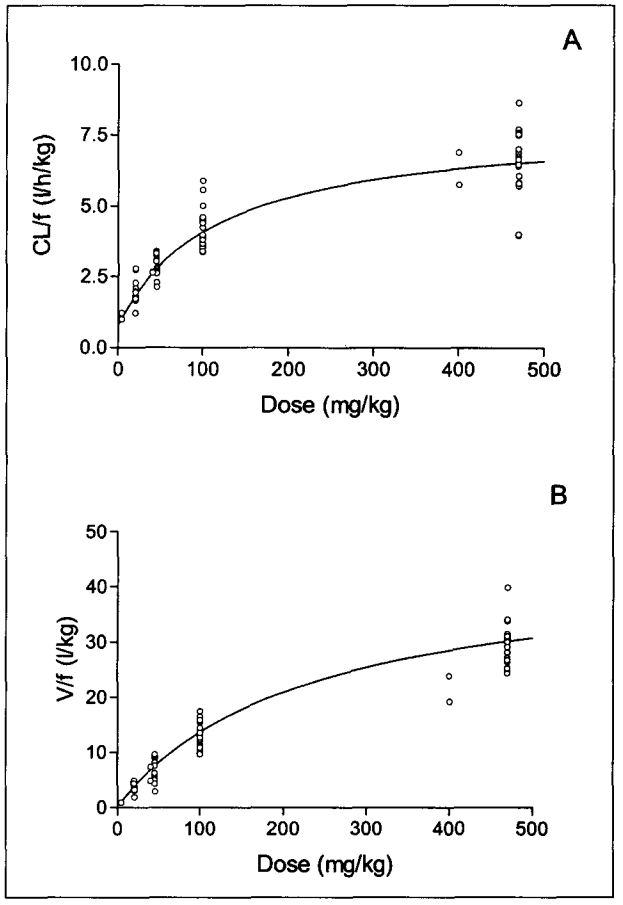

Fig. 2 : Individual estimates (O) and model predicted (line) values of CL/f versus dose (A) and V/f versus dose (B).

the value of the absorption rate constant (k_a) had to be fixed (a value of 10 h^{-1} was used for this purpose). Data file B was most sensitive in this respect. The results of the forward inclusion and of the backwards elimination were compared separately for the three data files. However the backwards elimination procedure was based on the same full model (Model No. 10) obtained using the complete data set disregarding the results of the forward inclusion using the reduced files.

As appears by inspection of Table II (forward inclusion of covariates), data file A would have given a full model without sex and time dependent CL/f and otherwise identical to the full model for the complete data file (Model No. 6 and 8 vs. 10). Furthermore forward inclusion using data file B would have resulted in a full model with non-linear dose dependency of V/f and otherwise identical to the model obtained using the complete data file (Model No. 5 vs. 10).

The backwards elimination procedure using data file A would have included sex dependent V/f and time dependent CL/f in addition to the covariates selected using the complete data file. The two additional covariate relations, however, would not have been included during forward inclusion. For data file B, backwards elimination retained the same covariates as the full data file (a sex dependency was observed for V/f, however $p<0.05$ and not as required $p< 0.001$).

DISCUSSION

This study shows that mixed effects modeling can be applied with success to toxicokinetic data in the rat even for a drug candidate, which exhibits dose-dependent kinetics. The population pharmacokinetic model seemed to describe the data adequately as measures of exposure (C_{max} and AUC) generated by the model matched those calculated by the NCA method well (Figure 4).

In contrast to population pharmacokinetic analyses of clinical data, where covariates initially are selected by methods such as multiple linear regression analysis or generalized additive modeling [9], the selection of covariates in toxicokinetic studies is fairly straightforward; and in our case limited to three possible covariates being: sex, dose and time (day of study). We used NONMEM for a stepwise (one by one) forward inclusion followed by backwards elimination of the covariates. This procedure was applied to study the effect of the covariates on clearance and volume of distribution (CL/f and V/f, respectively as the drug was administered orally). Covariate relationships were not analyzed for the absorption rate constant (k_a), as the interindividual variability was impossible to estimate for this parameter due to relatively few data points available from the absorption phase.

CL/f and V/f increased with dose exhibiting a saturation-like relationship (Figure 2). Considering the similar shape of the curves for CL/f and V/f, this could suggest that the fraction of the dose systemically absorbed (f) declined with increasing doses. This interpretation is supported by results from a separate study in rats demonstrating increased faecal excretion of radioactively labeled drug and a decreased fraction of DAB excreted unchanged (f_e) in the urine with increasing dose (f_e at 1.5, 20 and 470 mg/kg of DAB: 82%, 44% and 21% respectively - DAB is believed predominantly to be eliminated by renal excretion - Andersen, J.V. personal communication).

For Miglitol, an antidiabetic drug that structurally resembles DAB, dose dependent saturation of the absorption following oral administration has also been reported [10, 11]. Ahr et al., 1997 propose, that the poor oral bioavailability observed with high doses of Miglitol can be attributed to saturation of an active carrier-mediated

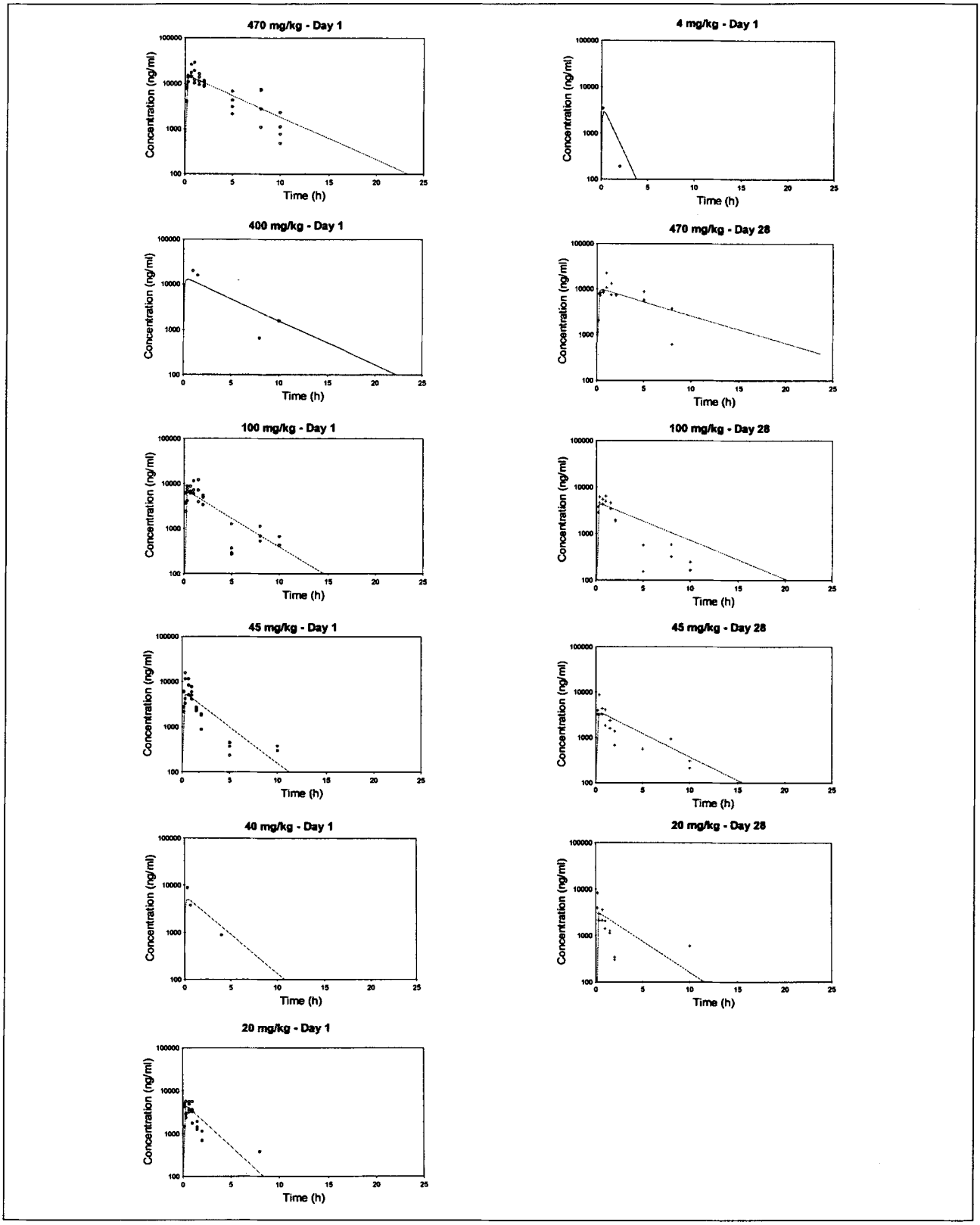

Fig. 3: Observed (O: Day 1; +: Day 28) and predicted (line) concentration-time profiles Day 1 and 28, respectively

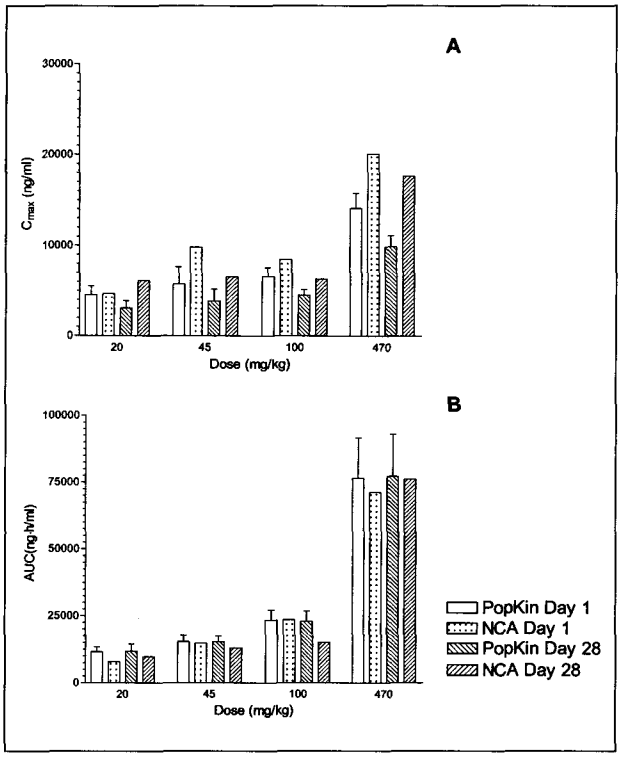

Fig. 4 : Mean C_{max} and AUC values calculated by non-compartmental analysis and population pharmacokinetics. Data from the population model are mean (±SD) empirical Bayes estimates. Data from the non-compartmental analysis are mean values from the two sexes.

transport mechanism located in the upper part of the small intestines [10]. Early Caco-2 studies have failed to demonstrate such a mechanism for DAB, but further experiments need to be performed.

Alternatively reduced absorption with increasing doses may be attributed to restrictions in the passive paracellular transport such as reported for Cimetidine [12]. Cimetidine is a small (MW: 252 g/mole), slightly basic (pK_a: 7.1), hydrophilic molecule with physicochemical characteristics similar to DAB, which also is highly water soluble (≥50 mg/ml), basic (pK_a: 8.2) and has a low octanol to water partition coefficient (logP: -1.2) [13]. Cimetidine has been chosen as a model drug for the "Class 3, low permeability - high solubility group" of compounds in the biopharmaceutical classification system [13]. This system has been proposed as a tool to correlate in vitro permeability and solubility characteristics with in vivo oral bioavailability. For "Class 3" compounds, the intestinal permeability is the rate-limiting step in the absorption process and the oral bioavailability of drugs in this group is often poor and highly variable [14]. In particular for Cimetidine, where passive paracellular diffusion is the principal route of traversing the intestinal mucosa, reduced (intestinal) permeability with increasing perfusion concentrations has been demonstrated in a rat intestinal perfusion model [12]. Gan et al., 1998 propose that H_2-receptor-antagonists have the potential to reduce the permeability of hydrophilic drugs across the intestinal mucosa by modulating the tight junctions causing a "tightening" of the intercellular junctions. For some of the H_2-receptor-antagonists a study in the Caco-2 model has demonstrated, that the passive diffusion of mannitol across the cell layer was decreased and the transepithelial resistance (TEER) increased and furthermore the transport of the drug itself across the Caco-2 cell monolayers was reduced in a concentration-dependent manner [15]. We might speculate if DAB could influence its own oral absorption by modulating the tightness of the tight junction complexes in a similar manner.

The final model contained separate dose dependent relationships for CL/f and V/f. We also investigated the alternative approach of modeling a dose dependent fraction systemically absorbed (f).
This was modeled as:

$$f = \theta_1 - \frac{\theta_2 \cdot Dose}{\theta_3 + Dose},$$

$$CL = \theta_4/f \text{ and } V = \theta_5/f$$

This model – by definition – resulted in a dose independent $t_{1/2}$ but the model fit was not as good as the final model presented here (details not shown).

On the other hand, if CL/f and V/f were solely dependent of the fraction absorbed the ratio of CL/f to V/f, representing the value of the elimination rate constant (k), should remain constant with increasing dose. However Figure 3 suggests that the plasma half-life ($t_{1/2}$) increased with increasing doses. Intravenous pharmacokinetic studies using a range of doses will have to be performed to clarify if a dose dependent clearance is present in rats.

A drawback of toxicokinetic studies as a source of information on the pharmacokinetic behaviour of a drug is the use of relatively high doses in such studies. This limitation can be overcome by adding data from other studies under conditions using lower doses to the data file. Thus, by adding data from a cardiovascular study of DAB in rats to the original toxicokinetic data file, we were able to expand the models of CL/f and V/f to a lower dose. This feature of non-linear mixed effects modeling is particularly important if the models are to be used for interspecies allometric scaling at therapeutic dose ranges [8].

The only other significant covariate besides dose was the effect of time on V/f. The value of V/f on study Day 28 was 55% higher than the value on Day 1 (Table III).

This effect was also apparent from the lower C_{max} values on Day 28 than on Day 1 (Figure 4, A). The explanation for this is not known. However, a similar effect was found in a other species (Ingwersen & Kiehr- unpublished observations). Long-term toxicity studies will reveal whether the observed time-dependent changes of V/f are consistent.

Our investigation of the data requirements for mixed effects modeling (Table II) showed that the number of blood samples could not be reduced from three to two samples per animal without affecting the outcome of the model building process. Thus, the results obtained during the covariate analysis were affected by the number of data points and this was most pronounced for data file A, where the sampling schedule on Day 1 and Day 28 were identical (staggered sampling, see Table I). Furthermore, the absorption rate constant could not be estimated in some of the modeling runs using the reduced data files, and this was most pronounced for file B. Thus, based on these observations, a sampling schedule of 2 samples per animal Day 1 and 1 sample per animal Day 28 seemed to be a minimum requirement.

In conclusion, we have confirmed that mixed effects modeling can be applied to toxicokinetic studies with sparse blood sampling and by merging with data files from other preclinical studies. These studies may be regarded as important sources of information on the pharmacokinetic properties of potential new drugs. In our opinion and in line with Bouzom et al. 2000, mixed effects modeling is considerably more laborious than noncompartmental analysis, which in most cases will continue to be the method of choice for quantification of exposure in toxicokinetics. However as shown in this study mixed effects modeling is feasible in toxicokinetic studies and it has the potential to enable PK/PD modeling and to simulate the outcome of future PK studies.

REFERENCES

1. Dahlem A.M., Allerheiligen S.R., Vodicnik M.J. (1995): Concomitant toxicokinetics: techniques for and interpretation of exposure data obtained during the conduct of toxicology studies. Toxicol. Pathol., 23, 170-178.
2. Van Bree J., Nedelman J., Steimer J.L., Tse F., Robinson W., Niederberger W. (1994): Application of sparse sampling approaches in rodent toxicokinetics: a prospective view. Drug. Inf. J., 28, 263-279.
3. Steimer J.L., Vozeh S., Racine-Poon A., Holdford N., O´Neill R. (1994): The population approach: Rationale, methods and application in clinical pharamacology and drug development. In Welling P.G and Balant L.P. (eds). Pharmacokinetics of Drugs. Editors. Berlin: Springer-Verlag.
4. Burtin P., Mentre F., Van Bree J., Steimer J.L. (1996): Sparse sampling for assessment of drug exposure in toxicological studies. Eur. J. Drug Metab. Pharmacokinet., 21, 105-111.
5. Gething P.A., Daley-Yates P.T. (1997): A sparse sampling mixed effects approach to toxicokinetic analysis. Drug Inf. J., 31, 521-527.
6. Meineke I., Eisele J., Certa H., Gundert-Remy U.M. (1998): Modeling of residual variability in toxicokinetic studies with sparse sampling: the case of tetrahydronaphtalene. Arch. Toxicol., 72, 807-810.
7. Bouzom F., Laveille C., Merdjan H., Jochemsen R.(2000): Use of nonlinear mixed effect modeling for the meta-analysis of preclinical pharmacokinetic data: application to S 20342 in the rat. J. Pharm. Sci., 89, 603-613.
8. Cosson V.F., Fuseau E., Efthymiopoulos C., Bye A. (1997): Mixed effect modeling of sumatriptan pharmacokinetics during drug development. I: Interspecies allometric scaling. J. Pharmacokinet. Biopharm., 25, 149-167.
9. Sun H., Fadiran E.O., Jones C.D., Lesko L., Huang S.M., Higgins K., Hu C., Machado S., Maldonado S., Williams R., Hossain M., Ette E.I. (1999): Population pharmacokinetics, a regulatory perspective. Clin. Pharmacokinet., 37, 41-58.
10. Ahr H.J., Boberg M., Brendel E., Krause H. P., Steinke W. (1997): Pharmacokinetics of Miglitol. Absorption, distribution, metabolism, and excretion following administration to rats, dogs, and man. Drug Res., 47, 734-745.
11. Steinke W. (1986): Intestinal absorption mechanism of the glucosidase inhibitor BAY m1099. Naunyn Schmiedebergs Arch., 332, R41.
12. Piyapolrungroj N., Zhou Y.S., Li C., Liu G., Zimmermann E., Fleisher D. (2000): Cimetidine absorption and elimination in rat small intestines. Drug Metab. Dispos., 28, 65-72.
13. Pade V., Stavchansky S. (1998): Link between drug absorption solubility and permeability measurements in Caco-2 cells. J. Pharm. Sci., 87, 1604-1607.
14. Amidon G.L., Lennernäs H., Shah V.P., Crison J.R. (1995): A theoretical basis for a biopharmaceutic drug classification: The correlation of in vitro drug product dissolution and in vivo bioavailability. Pharm. Res., 12, 413-420.
15. Gan L-S. L., Yanni S., Thakker D.R. (1998): Modulation of the tight junctions of the Caco-2 cell monolayers by H2-antagonists. Pharm. Res., 15, 53-57.