Nonlinear Mixed Effects Modeling of Single Dose and Multiple Dose Data for an Immediate Release (IR) and a Controlled Release (CR) Dosage Form of Alprazolam[1]

Mohammad Hossain,[2,5] Eugene Wright,[3] Raman Baweja,[2] Thomas Ludden,[2,4] and Raymond Miller[2]

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Purpose. NONMEM was applied to single dose and multiple dose bioavailability data for an immediate release (IR) and a controlled release (CR) dosage form of alprazolam to acquire additional information from the data which are not easily obtainable by traditional means.
Methods. The objective function value (OBJ) and diagnostic plots were used as measures of goodness of fit of the model to the data. A change in the OBJ value of 7.9 was necessary to show statistical significance ($p < 0.005$) between two models when the two models differed by 1 parameter.
Results. A two-compartment linear model with first-order absorption and elimination best describes the data. Including a lag time, two different rates of absorption (KA_{IR} and KA_{CR}), and bioavailability for the CR relative to the IR dosage form significantly improved the fit of the model to the data. Cigarette smoking was associated with a 100% increase in clearance of alprazolam as compared to non-smokers. The higher residual variability observed in this study, where interoccasion variability (IOV) was not initially modeled, could be explained to a large extent by the presence of significant interoccasion variability (IOV).
Conclusions. Since alprazolam has been suggested to be mainly metabolized by the CYP3A4 isozyme in humans, it appears that tobacco could be an inducer of CYP3A4 and/or alprazolam may be metabolized by other isozyme(s) (specifically, CYP1A1/1A2) that are induced by cigarette smoke. The population pharmacokinetic model approach combined with exploratory graphical data analysis is capable of identifying important covariates from well-controlled "data rich" Phase I studies early in drug development.

KEY WORDS: alprazolam; controlled release; relative bioavailability; NONMEM; P450; covariate analysis; interoccasion variability.

INTRODUCTION

Alprazolam is a triazolobenzodiazepine and its receptor binding characteristics are qualitatively similar to those of the other benzodiazepines (1). Alprazolam has been approved for the management of anxiety and panic disorders. The mean effective dose necessary to treat patients with panic syndrome is approximately 5 to 6 mg a day administered in divided doses, three or four times a day (2).

The drug is metabolized primarily by hepatic microsomal oxidation to at least 29 different metabolites (3), including two minor active metabolites, α-hydroxy-alprazolam and 4-hydroxy-alprazolam, which combined are usually less than 15% of the concentrations of the parent drug in human plasma (1,3). The pharmacokinetics of alprazolam are linear and concentrations are proportional to dose up to the maximum recommended dose of 10 mg a day (1).

The NONMEM (nonlinear mixed effect model) program has been used to estimate population pharmacokinetic parameters from routine clinical data involving sparse blood samples from large number of patients (4). In addition, the use of nonlinear mixed effects modeling has been shown to be useful in the assessment of routine bioavailability data (5). This approach may be particularly valuable and informative when other analyses may be confounded by the experimental conditions (6,7). A population pharmacokinetic approach may also be a useful tool for modeling Phase I human pharmacokinetic data as well as for identifying important covariates early in drug development. The objective of this current analysis was to simultaneously model single dose and multiple dose data for an immediate release (IR) and a recently developed controlled release (CR) dosage form of alprazolam (8) when given at different doses, and at different and unequal dosing intervals. The nonlinear mixed effects modeling approach using the NONMEM software (9) was specifically used: (i) to compare the bioavailability of alprazolam from an IR and a CR dosage form, (ii) to estimate the intersubject and random residual variability, (iii) to compare the intersubject variability between the IR and the CR dosage forms, (iv) to test the appropriateness of a first-order input rate versus a zero-order input rate for the CR dosage form, (v) to investigate the effect of available covariables (cigarette smoking, age, weight and gender) on alprazolam disposition in humans, and finally, (vi) to examine the effect of interoccasion variability (IOV) on population parameter estimates, interindividual variability and residual variability.

MATERIALS AND METHODS

Study Population

A total of 17 healthy adult subjects (7 females and 10 males) between the ages of 20 to 47 years (mean ± std: 31.8 ± 9.5 years) and weight ranging from 52 to 95 kg (mean ± std: 71.2 ± 10.5 kg) completed the bioavailability study. Eight of the subjects were cigarette smokers and nine were non-smokers. A total of 1289 measurable plasma concentrations were available for NONMEM analysis [approximately 76 samples (range: 74–76) per subject for all four treatments].

Study Design

The study was an open label, single dose and multiple dose study consisting of two, 2-way crossover designs. The research followed the tenets of the Declaration of Helsinki

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[2] Office of Clinical Pharmacology and Biopharmaceutics, Center for Drug Evaluation and Research, FDA 5600 Fishers Lane, Rockville, Maryland 20857.
[3] Clinical Pharmacokinetics Unit, The Upjohn Company, Kalamazoo, Michigan.
[4] Current Address: Department of Pharmaceutical Science, College of Pharmacy, University of Nebraska Medical Center, 600 South 42nd Street, Omaha, Nebraska 68198.
[5] To whom correspondence should be addressed.

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promulgated in 1964 and was approved by the Institutional Human Experimentation Review Board. Subjects provided written informed consent before enrollment in the study. The single dose treatments (1.5 mg for IR and 3 mg for CR) were administered to the subjects at 7:00 AM and multiple dosing began two days after the single dose treatments. The multiple dose treatment for the IR dosage form was administered at 7:00 AM, 12:00 NOON, 5:00 PM, and 10:00 PM (i.e., 1.5 mg QID using unequal dosing interval), and for CR dosage form was administered at 7:00 AM and 8:00 PM (i.e., 3 mg BID using unequal dosing interval) for 7 days. All treatments were administered with 180 ml of water and subjects were fasted from 10 hours before and up to 4 hours after the 7:00 AM doses. No caffeine containing beverages were consumed during the fasting period, and no fluids were allowed for 4 hours after the 7:00 AM doses. Due to the type of medication administered, subjects were allowed to remain sedentary.

Blood Sampling

A total of 14 blood samples of 7 ml each were drawn from an antecubital vein of each subject on Day 1 immediately before both single dose treatments (0 hour) and then at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, and 36 hours after the IR dose, and for the CR dose samples were collected at 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 30, 36, and 48 hours after the dose. Following multiple dose administration of the IR and CR dosage forms for 7 days, steady-state blood samples were drawn from each subject immediately before both multiple dose treatments (0 hour) on Day 7. Thirty blood samples from each subject were collected at 0.5, 1, 1.5, 2, 3, 4, 5 (12:00 NOON), 5.5, 6, 6.5, 7, 8, 9, 10 (5:00 PM), 10.5, 11, 11.5, 12, 13, 14, 15 (10:00 PM), 15.5, 16, 16.5, 17, 18, 19, 24, 36, and 48 hours after the IR dose and eighteen blood samples were drawn from each subject after the CR dose at 1, 1.5, 2, 3, 4, 6, 8, 12, 13, 13.5, 14, 15, 16, 18, 20, 24, 36, and 48 hours.

Analytical

Plasma alprazolam concentrations were measured using a modified HPLC method (10). Alprazolam and the internal standard (triazolam) were extracted from plasma and chromatographed under isocratic conditions. Peak heights were monitored with a variable wavelength ultraviolet detector at 223 nm and quantitation was achieved using peak height ratios. The recovery of alprazolam from plasma was 96 ± 4%. The interday coefficient of variation for alprazolam at the low end of the standard curve (1.0 ng/ml) was 2.5% and 3.2% at the upper end (100.0 ng/ml).

Pharmacostatistical Models

Structural Model

A two compartment open model (2-COM) parameterized in terms of clearance (CL), volume of central compartment (V1), intercompartmental clearance (Q), volume of peripheral compartment (V2), and first-order absorption rate constant (KA) using ADVAN4 TRANS4 from the PREDPP library of models provided in the NONMEM software (Version IV, Level 2) was used to fit the data. In addition, a zero-order input into the central compartment was also used to fit the data for the CR dosage form. This was compared with the first-order input rate to determine which input rate provided the best fit for the CR data. For relative bioavailability (F_{rel}), F was set to one for the IR dosage form (reference) and was estimated for the CR dosage form (test). All analyses were performed using the conventional first-order estimation method (Method = 0).

Statistical Model

Intersubject Variability. This was modeled using an exponential error model as follows:

$$CL_j = CL^*DEXP\eta_j^{CL};\ \eta_j^{CL}\ \text{i.i.d.} \sim N(0, \omega^2_{CL})$$

where CL_j is the hypothetical true total body clearance (CL) for the jth individual as predicted by the regression model. CL is the typical population value of clearance; the η_j^{CL} represents the persistent difference between the jth individual's CL value and that predicted by the regression model; η_j^{CL}'s are independent, identically distributed (i.i.d.) random variables with mean 0 and variance equal ω^2. Interindividual variability in V1, Q, V2, KA, T_{LAG} (lag time) and F_{rel} (relative bioavailability) were similarly modeled. KA was partitioned for the two dosage forms (KA_{IR} and KA_{CR}) using both a common variance or assigning different variances to the two different rates of absorption. Similarly, T_{LAG} was also partitioned (T_{LAG1} and T_{LAG2}). Interindividual variability in relative bioavailability (F_{REL} = CR/IR) was also modeled using an additive error model:

$$F_{RELj} = F_{rel} + \eta_j^{Frel};\ \eta_j^{Frel}\ \text{i.i.d.} \sim N(0, \omega^2_{F_{rel}})$$

Since, F_{rel} is expected to follow a standard normal distribution and is not necessarily limited to a value of 1, the simpler of the two models (i.e., the additive over the exponential error model) was chosen based on the pre-defined model selection criteria.

Residual Intrasubject Variability. A model involving a combination of constant coefficient of variation and additive error was used to model this component of variability as shown below:

$$C_{ij} = C_{mij} + C_{mij}^*\varepsilon_{1ij} + \varepsilon_{2ij}$$

where C_{ij} is the ith observed concentration for the jth individual; C_{mij} is the ith concentration predicted by the model for the jth individual. This value includes the contribution of the η^{CL} term. ε_1 and ε_2 are independent, identically distributed statistical errors with mean equal zero and variance equal σ^2. The magnitude of residual intraindividual variability usually depends on measurement, dosing, sampling and model misspecification errors but also on the presence of interoccasion variability (IOV) (11).

DATA ANALYSIS

Model Selection Criteria

The following criteria were used in selecting the optimized basic structural model and for evaluating the effect of adding a covariable in building the final regression model. In general, a lower value for the NONMEM objective function is desirable. When comparing two models (full vs reduced, e.g. 2-COM vs

1-COM) the chi-squared test ($p < 0.005$) of the log-likelihood difference (LLD) between the objective function values (OBJs) was used with degrees of freedom equal to the difference in number of parameters between the models. Thus, a change in the objective function value of 7.9 was necessary to show statistical significance ($p < 0.005$) between two models when the two models differed by 1 parameter. To partially compensate for the multiple comparisons, a conservative p value of less than 0.005 was chosen in the selection of a covariate (12). The goodness-of-fit of each NONMEM analysis was also assessed by the evaluation of the residual plots and by the decrease in the estimate of the inter-individual and residual variances. For non-hierarchical models, where all models had the same number of parameters, model comparison was based on examination of the OBJ, and other criteria enumerated above.

Model Building Steps

A combination of statistical techniques and graphical displays were used in the analysis of the multivariable data. A stepwise approach was used in the analysis of the data: (i) determination of a basic pharmacokinetic structural model using the NONMEM program and obtaining Bayesian individual parameter estimates (12–16). Empirical Bayes estimates of pharmacokinetic parameters derived from the two compartment (2-COM) open model fit of the data was obtained using the POSTHOC option within the NONMEM program, (ii) exploratory data analysis for a graphical screening of the relative importance of several covariates and their relationships with pharmacokinetic parameters were then examined (17) using S-PLUS statistical software package (Version 3.1) (18), and (iii) final NONMEM modeling to determine the relationship of subject demographics to pharmacokinetic parameters (12–16).

Covariates analyzed included total body weight, lean body mass, body surface area, cigarette smoking, age and gender. Each covariate was modeled linearly using all possible combinations and tested for a significant intercept term by fixing this value to zero and assessing the change in OBJ value in comparing that to a full model incorporating an intercept term. In order to diagnose the presence of IOV, the data set was modified so that each occasion for a subject was treated as if arising from a different subject. The difference in residual variability between the final covariate model and the final covariate model with IOV allows to extract the IOV contribution to total residual variability. The following equations (19) were used for calculation of lean body mass (LBM) and body surface area (BSA):

$$\text{LBM (men)} = 1.10 \times \text{TBW} - 120\ (\text{TBW/HT})^2$$

$$\text{LBM (women)} = 1.07 \times \text{TBW} - 148\ (\text{TBW/HT})^2$$

where TBW is total body weight in kg and HT is height in cm.

$$\text{BSA} = \text{WT}^{0.425} \times \text{HT}^{0.725} \times 0.007184$$

where WT is actual body weight in kg and HT is height in cm.

RESULTS

Initial NONMEM runs showed that the two compartment model with first-order absorption described the data (OBJ = 6856) better than a simple one compartment open model (OBJ = 6975). A combination of constant coefficient of variation (CCV) and additive residual error model (OBJ = 6646, model #1 in Table I) was found to describe the error in the data better than the CCV error model alone (OBJ = 6856) or the additive error model alone (OBJ = 6706.5). Partitioning of KA but assigning a common variance for the two different dosage forms (KA_{IR} and KA_{CR}) provided an OBJ of 5796.5 (LLD of −849.5 compared to model #1 in Table I, where KA was not partitioned). In addition, assigning different variances to the two different rates of absorption further reduced the OBJ value to 5746 (LLD of −50.5 compared to model #2 in Table I, where KA was partitioned but share a common variance). A common lag time (T_{LAG}) for the two dosage forms provided an OBJ of 5733 (LLD of −13 compared to the reduced model #3 in Table I). Partitioning of T_{LAG} (T_{LAG1} and T_{LAG2}) and assigning either a common variance or two different variances for the two different dosage forms (IR and CR) did not improve the fit. Finally, including relative bioavailability (F_{REL} = CR/IR) significantly improved the fit with an OBJ of 5630.5 (LLD of −102.5 compared to the reduced model #4 in Table I), and provided the final 2-COM optimized structural model for covariate analysis (model #5 in Table I).

Exploratory graphical data analysis indicated possible relationships between clearance and total body weight, clearance and smoking, and volume of peripheral compartment and total body weight. Using the final, optimized 2-COM structural model #5 of Table I, linear models for total body weight (WT) as a predictor of relevant model parameters (specifically, CL, V1, Q, and V2) provided an OBJ value of 5597 (LLD of −33.5 from optimized structural model). On further testing, using all possible combinations of WT on CL, V1, Q and V2, WT was only found to be a predictor of CL and V2 (OBJ = 5598), and the intercept term was found to be unnecessary for modeling these parameters (OBJ = 5598.5, model #6 in Table II).

NONMEM analysis confirmed that cigarette smoking was a very important predictor of alprazolam clearance. Using all possible combinations of CIG on CL, V1, Q, V2, and KA, CIG was only found to be a predictor of CL (OBJ = 5350.5) with a loglikelihood difference of −248 between the full (model #7) and the reduced model (model #6) shown on Table II. Lean body mass (LBM) was a better predictor of clearance (CL) as well as the peripheral volume of distribution (V2) of alprazolam than WT or body surface area (BSA), while age was found not to be a predictor of CL and V2 (Table II). Gender was found not to be a significant predictor of clearance of alprazolam (Table II).

The final covariate model (model #10 in Tables II and III) was reanalyzed using a zero-order input rate for the sustained release dosage form. The magnitude of residual variability remained unchanged at 34%, however, the OBJ value increased from 5337.5 to 5454.5 suggesting a first-order input rate is more appropriate for the CR dosage form. Figure 1a shows the relationship between the observed and predicted alprazolam concentrations using the optimized 2-COM structural model described in Table I (model #5), while Figure 1b shows a much more improved fit between the observed and predicted alprazolam concentrations using the final covariate model (model #10) described in Tables II and III. In Figures 1a and 1b, the observed higher concentrations represent steady-state concentrations following administration of IR and CR dosage forms in two individuals and these concentrations constitute a very small fraction of the total data set.

Table 1. Optimization of the Two Compartment Structural Model

	Structural models										
No.	CL	V1	Q	V2	KA	T_{LAG}	F_{REL}	OBJ[d]	cf[e]	df[f]	LLD[g]
1	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	—	—	6646	—	—	—
2	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	$2/1\eta^b$	—	—	5796.5	1	1	-849.5^h
3	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	$2/2\eta^c$	—	—	5746	2	1	-50.5^h
4	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	$2/2\eta^c$	$1/1\eta^a$	—	5733	3	2	-13^h
5	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	$1/1\eta^a$	$2/2\eta^c$	$1/1\eta^a$	$1/1\eta^a$	5630.5	4	2	-102.5^h

[a] No partitioning ($1/1\eta$).
[b] Partitioning of a parameter and having a common variance ($2/1\eta$).
[c] Partitioning of a parameter and associated variance for two dosage forms, IR and CR ($2/2\eta$).
[d] Objective function value.
[e] Compared with.
[f] Degree of freedom.
[g] Log-likelihood difference.
[h] Statistically significant at $p < 0.005$.

Table 2. Effect of Cigarette Smoking (CIG), Age, Lean Body Mass (LBM), Body Surface Area (BSA) and Gender (GEN) on Structural Model Parameters

	Covariate Models Tested										
Model no.	CL $1/1\eta$	V1 $1/1\eta$	Q $1/1\eta$	V2 $1/1\eta$	KA $2/2\eta$	T_{LAG} $1/1\eta$	F_{REL} $1/1\eta$	OBJ	cf	df	LLD
6	WT	—	—	WT	—	—	—	5598.5	5	0	-32^a
7	WT + CIG	—	—	WT	—	—	—	5350.5	6	1	-248^a
	Weight (WT) Replaced by AGE, Body Surface Area (BSA) or Lean Body Mass (LBM)										
8	AGE + CIG	—	—	AGE	—	—	—	5609	7	0	$+258.5^a$
9	BSA + CIG	—	—	BSA	—	—	—	5376.4	7	0	$+25.9^a$
									10	0	$+38.9^a$
10	LBM + CIG	—	—	LBM	—	—	—	5337.5	7	0	-13^a
11	LBM + CIG + GEN	—	—	LBM	—	—	—	5336	10	1	-1.5

[a] Statistically significant at $p < 0.005$.

Table 3. Typical Population Parameters and Percent Relative Standard Error (%RSE)

Phase I study	Estimate	CL/F (l/hr)	V1/F (l)	Q/F (l/hr)	V2/F (l)	KA_{IR} (1/hr)	KA_{CR} (1/hr)	T_{LAG} (hr)	F_{REL}	Residual variability
		CL/F = 4.5 l/hr			V_β/F = 67.5 l		KA_{IR} = 1.3 hr^{-1}		F_{REL} = 1.0	
2-COM	Estimate	3.88	21.7	52.7	49.5	1.59	0.167	0.223	1.2	23%-10 ng/ml
No covariate (Model #5)	%RSE	11%	33.5	19.5%	12%	32.5%	12%	3%	14%	35%-5 ng/ml
2-COM final model with covariate[a]	Estimate	3.77[c] 7.5[d]	20.2	57.5	64.5	1.74	0.17	0.225	1.08	22%-10 ng/ml
(Model #10)	%RSE	10%[c] 23.5%[d]	29%	21%	11%	29%	9%	3%	7.5%	34%-5 ng/ml
2-COM Final model with covariate[a,b]	Estimate	4.28[c] 7.93[d]	22.7	59.7	68.2	1.57	0.202	0.231	1.13	13%-10 ng/ml
(IOV Diagnosis)	%RSE	6%[c] 19%[d]	28.5%	18%	9.5%	33.5%	16.5%	2%	9%	15%-5 ng/ml

[a] Typical population parameters for the final covariate model were calculated using an average adult weight of 70 kg.
[b] For the diagnosis of IOV, each subject was coded as a separate individual for each occasion and the modified data set was re-analyzed using the final covariate model.
[c] Non-smokers.
[d] Smokers.

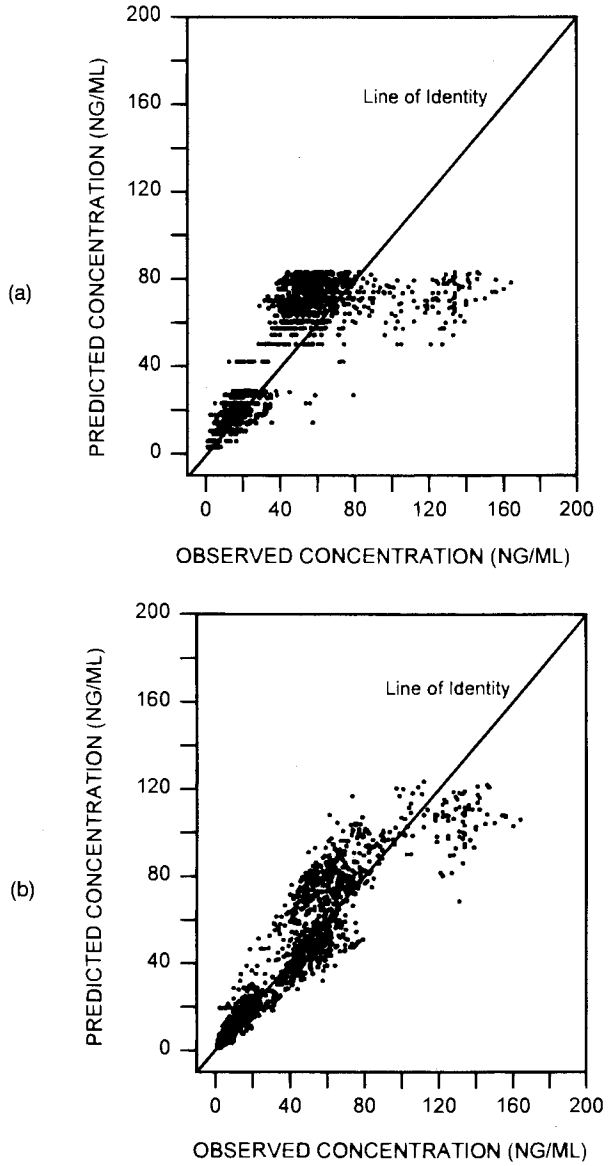

Fig. 1. (a) Optimized two compartment structural model. (b) Final two compartment covariate model.

LBM and CIG helped to explain the intersubject variability in CL which was decreased from 38% (optimized base model #5) to 21.5% (final covariate model #10) (Table IV). However, the magnitude of residual variability remained unchanged at 34% for alprazolam plasma concentration of 5 ng/ml (Table III). Thus, we attempted to diagnose the presence of IOV, without actually fitting an IOV model to the data set. The final covariate model (OBJ = 5337.5, model #10 in Tables II and III) was re-analyzed using the same data set, which was modified so that each subject was coded as a different individual for each occasion (11). The OBJ value was lower (OBJ = 5146.5) and residual variability decreased to 15% for alprazolam plasma concentration of 5 ng/ml, thereby, confirming the presence of IOV (Table III). However, as expected, an increase in intersubject variability of 4.5–13.5% was observed in some parameters (i.e., CL, Q and V2), except one (i.e., KA) where it was lower by 9% (Table IV). Once the existence of IOV was confirmed, the modified data set that was created by coding each subject as a different individual for each occasion was then used with the final covariate model (model #10 in Tables II and III), to test whether any of the earlier significant covariables were erroneously selected. This was tested by stepwise deletion of each of the covariate. All earlier selected covariables were found to be significant, which is consistent with the final covariate model (model #10 in Tables II and III).

DISCUSSION

The estimated interindividual variability associated with KA_{CR} (CV = 17%) was much lower than with KA_{IR} (CV = 39%) in the optimized structural model (model #5 in Table IV). An F_{REL} of 1.08 for the CR dosage form (Table III) is consistent with values reported in the Phase I study utilizing the standard two-stage (STS) approach.

Gender was found not to be a significant predictor of clearance of alprazolam and is consistent with literature reports (20). Cigarette smoking was associated with a 100% increase in clearance of alprazolam as compared to non-smokers (final covariate model #10 in Table III). This result differs from a previous report showing an increase in alprazolam clearance of only 25% in smokers which did not reach statistical significance (21). However, the sample size in that study was small (n = 5). The estimated population parameters are comparable to the corresponding Phase I estimates (Table III), however, the interindividual variability was generally reduced by the inclusion of covariables (model #10 in Table IV).

The effect of tobacco smoking on alprazolam pharmacokinetics is demonstrated by the present analysis. Since alprazolam has been suggested to be mainly metabolized by the CYP3A4 isozyme based on several *in vitro* and *in vivo* drug interaction studies (1,3,22,23), an explanation could be that tobacco may be an inducer of CYP3A4 (24,25) and/or alprazolam may be metabolized by other isozyme(s) (specifically, CYP1A1/1A2) (23) that are induced by cigarette smoke (24–28). This needs further investigation. It is possible that drugs which are primarily metabolized by a specific P450 isoform could also concurrently be metabolized by multiple P450 pathways. Fluvoxamine, an inhibitor of CYP1A2 (29,30), has been reported to inhibit the 4- and α-hydroxylation of alprazolam *in vitro* (31) and to increase plasma alprazolam concentrations *in vivo* in humans (32). Erythromycin, an inhibitor of CYP3A4, has been shown to inhibit the metabolism of alprazolam in humans (23). In addition, erythromycin is also known to inhibit the metabolism of theophylline (33), which is a substrate of CYP1A2 (30,34). These findings suggest that the inhibitory effect of erythromycin on the metabolism of alprazolam may be mediated partly through CYP1A2. Using liver microsomes from adult male mice previously exposed to cigarette smoke, enzymatic activity determinations for erythromycin N-demethylase activity known to be catalyzed by CYP3A has been shown to increase by up to 95%, and Western blot analysis revealed about a 2 fold increase in CYP3A levels (24). If these findings could be extrapolated to humans, then the observed 100% increase in clearance of alprazolam in cigarette smokers would be consistent with the magnitude of hepatic CYP3A induction reported in mice, and would also support the present belief that alprazolam is primarily metabolized by CYP3A. Currently, CYP3A1/3A2 are the major P450 3A subfamily identified in the rat (35). These

Table 4. Variance Estimates (EST) and Percent Relative Standard Error (%RSE)

Phase I study		ω^{CL}	ω^{V1}	ω^{Q}	ω^{V2}	ω^{KA}_{IR}	ω^{KA}_{CR}	ω^{T}_{LAG}	ω^{F}_{REL}
	%RSE			CL = 32%	V_β = 18%		F_{REL} = 16%		
2-COM No covariate (Model #5)	EST	0.144	0.147	0.157	0.02	0.154	0.03	0.004	0.053
	%RSE	38%	38.5%	40%	14.5%	39%	17%	6%	19%
2-COM Final model with covariate (Model #10)	EST	0.046	0.149	0.186	0.01	0.185	0.05	0.003	0.011
	%RSE	21.5%	38.5%	43%	10%	43%	22%	5.5%	10%
2-COM Final model with covariate (IOV Diagnosis)[a]	EST	0.068	0.136	0.321	0.026	0.176	0.047	0.008	0.00006
	%RSE	26%	37%	56.5%	16%	42%	21.5%	9%	1%

[a] For the diagnosis of IOV, each subject was coded as a separate individual for each occasion and the modified data set was re-analyzed using the final covariate model.

forms of P450 3A in the rat have been specifically shown to be responsible for erythromycin N-demethylation, which in humans has been shown to be catalyzed by CYP3A3/3A4 (26). It is possible that a substrate metabolized by a specific P450 enzyme in an animal model (e.g., CYP3A1/3A2 in the rat) is metabolized by a structurally different P450 enzyme in humans (e.g., CYP3A3/3A4).

It has been reported that the presence of unrecognized interoccasion variability (IOV) on the random effect parameter estimates can cause biases by overestimation of the residual error in NONMEM (11). The population parameter estimates and associated percent relative standard errors (Table III), and variance parameter estimates and associated percent coefficients of variation (interindividual variability) (Table IV) were comparable for the final covariate model, when analyzed with or without treating each subject as a separate individual for each occasion for the diagnosis of IOV. By extracting the IOV contribution to residual variability (19% at 5 ng/mL, i.e., the difference between the residual variability of final covariate model #10 and final covariate model with IOV), what remains (15%) is probably closer to "true" residual error, representing variability due to measurement, dosing, sampling and model misspecification errors (Table III).

CONCLUSIONS

The population pharmacokinetic model approach combined with exploratory graphical data analysis is capable of identifying important covariates from well-controlled "data rich" Phase I studies early in drug development. It is probably beneficial to have diverse demographics in Phase I pharmacokinetic studies so that an initial screening of important covariates could be conducted without sacrificing the primary objective of such a study. The nonlinear mixed effects modeling approach appeared to be a useful tool for the efficient, simultaneous modeling of single and multiple dose data for both IR and CR dosage forms. However, one should be cautious in applying NONMEM during traditional model analysis because the presence of unrecognized IOV could cause biases by overestimation of the residual error. The use of NONMEM allowed appropriate and informative analysis of routine bioavailability data.

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