Population Pharmacokinetics of Tirilazad: Effects of Weight, Gender, Concomitant Phenytoin, and Subarachnoid Hemorrhage

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Purpose. Data collected during Phase I and II in the development of tirilazad were pooled and analyzed using nonlinear mixed effects models to assess covariates which might affect tirilazad pharmacokinetics.
Methods. Four single dose and five multiple dose studies in normal volunteers were combined with two multiple dose studies performed in patients with subarachnoid hemorrhage (SAH) to identify factors related to intersubject variability in clearance (*CL*) and central compartment volume (*Vc*). Data from 253 subjects, which consisted of 7,219 tirilazad concentrations, were analyzed. The effects of weight, gender, patient versus volunteer status, and phenytoin use were evaluated.
Results. Relative to male volunteers not receiving concomitant phenytoin, significant effects on clearance included: a 46% increase in volunteers receiving phenytoin, and an 82% increase in clearance associated with SAH patients (all of whom received phenytoin). Significant effects on *Vc* were: a 26% increase for female volunteers not receiving phenytoin, a 12% decrease for volunteers receiving concomitant phenytoin, a 152% increase for male SAH patients, and a 270% increase for female SAH patients. Incorporating patient covariate effects substantially reduced the interindividual variability (from 27.9% to 24.7% for clearance and from 48.2% to 37.5% for *Vc*). Residual variability was estimated at 66% coefficient of variation (CV) in SAH patients and at 22–48% CV over the range of predicted concentrations in normal volunteers.
Conclusions. The most important factors affecting tirilazad pharmacokinetics are the administration of phenytoin (increased *CL*) and SAH (increased *Vc* and residual variability). The effect of gender on tirilazad pharmacokinetics was modest.

KEY WORDS: population pharmacokinetics; tirilazad; subarachnoid hemorrage; gender effect; enzyme induction.

INTRODUCTION

Tirilazad is a membrane lipid peroxidation inhibitor which is active in animal models of neuronal damage due to ischemia and reperfusion (1). Tirilazad has been evaluated clinically in neurological trauma, but most extensively in the management of subarachnoid hemorrhage (SAH) (2,3).

Tirilazad pharmacokinetics are approximately linear over the dosage range 1.0–16.0 mg/kg/day. Steady-state is not achieved within the 10 day dosing period, due to the long half-life of the drug (4). Tirilazad is primarily eliminated via metabolism by CYP3A family of cytochrome P450 (5). Compounds which induce these enzymes, such as phenytoin (6,7) and phenobarbital (8) have been shown to enhance the clearance of tirilazad, while the CYP3A inhibitor ketoconazole dramatically inhibits tirilazad clearance (9). Tirilazad clearance is apparently faster in women than men (10,11) and is reduced approximately 30% in the elderly (10). Plasma concentrations of tirilazad appear to be lower in SAH patients (unpublished data) and head injury (12) than those observed in healthy volunteers; this effect may be due to use of enzyme-inducing anticonvulsants.

The European-Australasian SAH study (3) showed a reduction in mortality at 76 days at a dose of 6 mg/kg/day tirilazad in male, but not female, SAH patients. The lack of effect in females has been hypothesized to be due to the gender effect on tirilazad pharmacokinetics. However, pharmacodynamic differences may also contribute to this effect. The lack of a robust mortality reduction in the North American SAH study (in which 70% of the patients received phenytoin) (13), suggests that diminished response is observed in the presence of enzyme-inducing anticonvulsant use. Taken together, these results suggest that effects on tirilazad pharmacokinetics may significantly influence successful management of SAH patients.

A number of pharmacokinetic studies have addressed the basic pharmacokinetic properties of tirilazad and the impact of various factors on tirilazad pharmacokinetics (4,8,10,11,14–17). These studies have been relatively small and have used various dosing and sampling strategies. This has resulted in a fragmented picture of tirilazad pharmacokinetics, particularly with respect to age and gender, and has precluded an investigation of the multivariable effects of age, gender and phenytoin on tirilazad pharmacokinetics.

To overcome theses problems, a population analysis of sparse data from Phase III trials would normally be conducted. However, initial evaluations (unpublished data) indicated that sparse sampling strategies alone would not be effective for studying tirilazad pharmacokinetics due to the multiexponential character of the drug's plasma concentration-time profile. These same investigations indicated that a four-compartment model is necessary for population pharmacokinetics for tirilazad, as the objective function dropped 1100 points in going from a three- to four-compartment model. This complex pharmacokinetic model results in some difficulty in collecting data during some of the trials. In those trials involving patients, it has been difficult to collect quality data due to early death, poor adherence to protocol specified infusion and sampling protocols, and apparent problems with the accurate recording of the timing of study events. These factors combine to make a population analysis of data from Phase II efficacy sparse sampling strategies logistically infeasible.

The present analysis described in this report was performed using available tirilazad plasma concentrations across a number of formal pharmacokinetic studies and observational studies of patients. This analysis was performed to allow a more comprehensive evaluation of weight, gender, concomitant phenytoin and SAH effects on tirilazad pharmacokinetics.

METHODS

Study Design

A summary study design, dosing and sampling strategies for the studies included in this analysis is provided in Table 1.

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Table 1. Characteristics of the Eleven Studies Used for the Population Pharmacokinetic Analysis of Tirilazad

Study (reference)	Subjects (normal/SAH)	Number/ gender	Study duration and dose	Concomitant treatment	PK days (extensive sampling)	Intervening troughs
3 (14)	Normal	26 Males	5.25 days 0.5, 1, 2, 4, 6 mg/kg/ day	None	1 and 6	Yes
22 (10)	Normal	23 11M, 12F	Single Dose 1.5 and 3 mg/kg	None	1	Not Applicable
33 (18)	Normal	14 Males	Single Dose 0.5, 1.5, 3 mg/kg	None	1	Not Applicable
34 (4)	Normal	37 Males	10.25 days 1, 3, 6 mg/kg/day 5.25 days 10 mg/kg/day	None	1, 6, and 11	Yes
39 (6)	Normal	21 Males	5.25 days 6, 10 mg/kg/day	Phenytoin	1 and 6	Yes
40 (19)	Normal	7 5M, 2F	Single Dose 2 mg/kg	None	1	Not Applicable
51 (11)	Normal	30 8M, 22F	Single Dose 3 mg/kg	None	1	Not Applicable
52 (22)	Normal	11 6M, 5F	7.25 days 6 mg/kg/day	Phenytoin	1 and 8	Yes
71 (20)	Normal	23 17M, 6F	7.25 days 6, 12, 16 mg/kg/day	None	1 and 8	Yes
25 (unpublished data)	SAH	7 2M, 5F	7–10 days 2 mg/kg/day	Phenytoin/ Phenobarbital	1, 5, and 7–10	Yes
55 (unpublished data)	SAH	54 17M, 37F	7–10 days 6, 10, or 15 mg/kg/day	Phenytoin/ Phenobarbital and Nimodipine	7–10	Yes

All doses were administered as ten minute infusions. In healthy volunteer studies blood samples after single doses were collected immediately prior to dosing and up to 168 hours following administration. Multiple doses ranging from 0.125 to 4.0 mg/kg were administered every 6 hours; daily trough levels were obtained on the intervening days between the full kinetic profile sampling days. In study 25, involving SAH patients, a maximum of four post-dose plasma concentrations were obtained at 1, 3, and 5 hours post-dose and immediately following the infusion. In study 55, trough tirilazad concentrations were measured and a full profile was measured following the last dose.

Plasma tirilazad was quantified by specific high-performance liquid chromatographic (HPLC) methods. The reader is directed to the individual study references for more detail concerning the assays used (4,14,15). For all assays, the coefficients of variation (CV) for quality control standards were $\leq 7.0\%$.

NONMEM Analysis

The plasma concentration-time data were fit to a four-compartment, mammillary model using the NONMEM computer program, version IV (ADVAN7 subroutine), with first order estimation implemented (21). The following parameters were estimated: systemic clearance (*CL*), central compartment volume (*Vc*), volumes of the peripheral compartments (V_2, V_3, V_4), and intercompartmental clearances (Q_{12}, Q_{13}, Q_{14}).

Interindividual variability in *CL* and *Vc* was modeled using an exponential error term:

$$CL_j = \tilde{C}L_j \cdot e^{\eta_j^{CL}} \quad (1)$$

$$Vc_j = \tilde{V}c_j \cdot e^{\eta_j^{Vc}} \quad (2)$$

where:

CL_j, Vc_j, = the true value of *CL* and *Vc* in the j^{th} patient, respectively;

$\tilde{C}L_j$, $\tilde{V}c_j$, = the typical value of *CL* and *Vc* in the j^{th} patient, respectively; and

η_j^{CL} = the persistent difference between the true value of *CL* in the j^{th} patient and the predicted value; the

η_j^{CL} = are independent, identically distributed statistical errors with a mean of 0 and a variance equal to ω^{CL^2}. The interpretation of the model for interindividual variability in *Vc* is analogous to that of *CL*.

This model assumes constant variance with respect to the log of the typical value of the pharmacokinetic parameter, and the estimates are presented as percent CVs. A term estimating the covariance between the random effect parameters for *CL* and *Vc* was also estimated.

Residual variability, was modeled using a combination additive plus constant CV error model as shown in Eq. 3.

$$Cp_{ij} = \hat{C}p_{ij} + \varepsilon_{1ij} + \hat{C}p_{ij} \cdot \varepsilon_{2ij} \quad (3)$$

where:

$\hat{C}p_{ij}$ = the i^{th} plasma concentration in the j^{th} patient predicted using the specified model;
Cp_{ij} = the measured value of the i^{th} plasma concentration in the j^{th} patient;
ε_{1ij} = random variable representing the additive component of residual variability; and
ε_{2ij} = random variable representing the constant CV (proportional) component of residual variability.

Using this error model, the variance of the difference between the measured and predicted concentrations can be expressed using Equation 4.

$$\text{var}(Cp_{ij} - \hat{C}p_{ij}) = \sigma_1^2 + \sigma_2^2 \cdot (\hat{C}p_{ij})^2 \quad (4)$$

where:

σ_1^2 = variance of ε_{1ij}, which represents the additive component of residual variability; and
σ_2^2 = variance of ε_{2ij}, which represents the constant CV (proportional) component of residual variability.

In addition, an indicator variable distinguishing patients with SAH from healthy volunteers was included in the residual variability model, thus allowing separate estimates of σ_1^2 and σ_2^2 for the two populations. The full model was implemented as shown below in Eq. 5.

$$Cp_{ij} = \hat{C}p + \varepsilon_{1ij} \cdot SAH_j + \hat{C}p_{ij} \cdot \varepsilon_{2ij} \cdot SAH_j + \varepsilon_{3ij} \cdot (1 - SAH_j) + \hat{C}p_{ij} \cdot \varepsilon_{4ij} \cdot (1 - SAH_j) \quad (5)$$

where:

SAH_j = an indicator variable for the j^{th} subject with a value of 1 if the subject represents a patient with SAH and 0 if the subject is a normal volunteer;
ε_{1ij}, ε_{2ij} = random variables representing the discrepancy between the i^{th} measured plasma concentration and the corresponding predicted concentration in the j^{th} SAH patient; and
ε_{3ij}, ε_{4ij} = random variables representing the discrepancy between the i^{th} measured plasma concentration and the corresponding predicted concentration in the j^{th} normal volunteer.

Covariate Analyses

The influence of each of the following patient covariates was tested on *CL* and *Vc*: weight, gender, concomitant phenytoin and patient versus volunteer status. Weight was evaluated using a centered, linear model as shown below in Eq. 6.

$$\tilde{C}L_j = \Theta_{CL}^{int} + (WT_j - \overline{WT}) \cdot \Theta_{CL}^{wt} \quad (6)$$

where:

WT_j = the body weight in kilograms of the j^{th} subject;
$\overline{WT}$ = the mean weight for all subjects in the population;
Θ_{CL}^{int} = the intercept of the clearance-weight relationship or the typical value of clearance for a patient weighing the population mean weight; and
Θ_{CL}^{wt} = the slope of the clearance-weight relationship.

For patient versus volunteer status and concomitant phenytoin assessment, three subpopulations were identified: normal volunteers not receiving phenytoin, normal volunteers receiving phenytoin, and SAH patients receiving phenytoin. Within each of these subpopulations, an effect of gender allowed for the estimation of two separate subpopulations, for a total of six distinct subpopulation effects on *CL* and *Vc*. For example, the following model shown in Eq. 7 was used to evaluate the clearance of female normal volunteers receiving phenytoin as compared to all other populations.

$$\tilde{CL}_j = \phi_{CL} \cdot (1 + \Theta_{CL}^{pop} \cdot SEXF_j \cdot (1 - SAH_j) \cdot PHT_j) \quad (7)$$

where:

ϕ_{CL} = the typical value of *CL* for populations other than female, normal volunteers receiving phenytoin, accounting for weight as shown in Equation 6;
Θ_{CL}^{pop} = the mean fractional increase or decrease in *CL* associated with the particular subpopulation defined by the covariate terms;
$SEXF_j$ = an indicator variable for gender, with a value of 1 if the j^{th} subject is female and 0 if the j^{th} subject is male;
SAH_j = an indicator variable for patient versus volunteer status, with a value of 1 if the j^{th} subject is a patient with subarachnoid hemorrhage and 0 if the j^{th} subject is a normal volunteer; and
PHT_j = an indicator variable for concomitant phenytoin, with a value of 1 if the j^{th} subject received phenytoin and 0 if the j^{th} subject did not receive phenytoin.

A backward elimination procedure was performed on the full model by successively fixing covariate values to the null hypothesis value and re-estimating the model. If there was no statistically significant difference in the fit to the model when a particular effect was deleted, the effect was dropped.

Statistical Analysis

Statistical significance was assessed by the change in the log likelihood value obtained for various models. For each analysis, NONMEM computes the minimum value of the objective function, a statistic which is proportional to minus twice the log likelihood of the data. In order to retain only those variables with large contributions in the final multivariable model, a change of at least 6.63 ($\alpha = 0.01$, 1 degree of freedom) was required to reach statistical significance for the retention of a single parameter in the multivariable model during the backward elimination phase.

The goodness-of-fit of each NONMEM analysis was also assessed by the examination of scatterplots of predicted versus measured plasma concentrations and weighted residuals, the percent standard errors of the mean (%SEM = standard error/parameter estimate * 100%) of parameter estimates, and

changes in the estimates of interindividual and residual variability resulting from the inclusion/deletion of parameters in the regression formulas given above.

NONMEM Dataset Construction

A total of 316 subjects with 9,052 concentrations were pooled from the 11 studies for population analysis. Several small subgroups of subjects were deleted from the database for the following reasons: (i) small size of the subpopulation, (ii) known or potential effect of subjects with the particular characteristic to influence tirilazad clearance, and (iii) inclusion of the subgroup was not central to the objectives of this analysis. The deleted subgroups were as follows: cirrhotics (7 subjects with 154 concentrations); black, hispanic, and asian subjects (28 subjects with 906 concentrations); subjects receiving phenobarbital (16 subjects with 652 concentrations) and SAH patients not receiving phenytoin (9 subjects with 79 concentrations). These groups were removed to decrease overall variability and because reliable estimates of effects in these groups could not be obtained (due to limited subject numbers). In preliminary data exploration, a number of concentrations were identified with apparent recording errors relative to timing of the last dose or sample collection. For example, based on the time since last dose a sample should have been a trough, but was in fact, a peak concentration. A total of 42 samples were deleted from the dataset for this reason. Thus, the database for analysis consisted of 253 subjects and 7,219 concentrations.

RESULTS

Database Description

Table 2 summarizes the categorical and continuous patient demographic factors in the database, stratified by subject type. Note the differences in the characteristics of the patient and healthy volunteer populations. The patients tend to be older than the volunteers and there is a much higher percentage of female patients than female volunteers. In addition, all patients in this population were receiving concomitant phenytoin while only 16% of the healthy volunteers studied in the drug-drug interaction studies received concomitant phenytoin.

A total of 19 (15 males and 4 females) normal volunteers enrolled in studies 39 and 52 (n = 9 and n = 10, respectively) received concomitant phenytoin during only one treatment arm. Therefore, each concentration record in the database was coded separately with regard to whether or not it was measured in the presence of concomitant phenytoin. Due to the induction of tirilazad clearance within 48 hours of commencing phenytoin administration (6), only those tirilazad concentrations measured more than 48 hours after the initiation of phenytoin therapy were retained in the database.

Table 2. Summary of Categorical and Continuous Patient Demographic Factors

Patient factor	Normal volunteers (N = 192)	Subarachnoid hemorrhage patients (N = 61)	Entire population (N = 253)
Gender			
n (%) female	47 (24.5%)	42 (68.9%)	89 (35.2%)
n (%) male	145 (75.5%)	19 (31.1%)	164 (64.8%)
Concomitant phenytoin			
n (%) yes	31 (16.1%)	61 (100%)	92 (36.4%)
n (%) no	161 (83.9%)	0 (0%)	161 (63.6%)
Age (years)			
mean (SD)	36.9 (15.2)	51.8 (14.0)	40.5 (16.2)
range	18–86	23–79	18–86
Weight (kg)			
mean (SD)	75.0 (11.1)	76.7 (15.9)	75.4 (12.4)
range	47.4–108.2	48.3–118.5	47.4–118.5

Model Development

Figure 1 shows the measured tirilazad concentrations plotted against time since last dose for representative subjects. The pharmacostatistical model, combining a four-compartment structural model, random effect parameters modeled using Eqs. 1–3, and a covariance term between *CL* and *Vc* was evaluated on this dataset. This structural model was found to fit these data reasonably well. A significant improvement in the model was obtained when the effect of subject type (patient versus volunteer) was incorporated into the residual variability model as shown in Eq. 5. However, the additive component of residual variability for the SAH patients was estimated to be nearly zero and could be removed from the model with no detriment to the fit. Therefore, the basic structural model consisted of a constant CV residual variability component for SAH patients and a combination additive plus constant CV component for the normal volunteers. Table 3 summarizes the parameter, standard

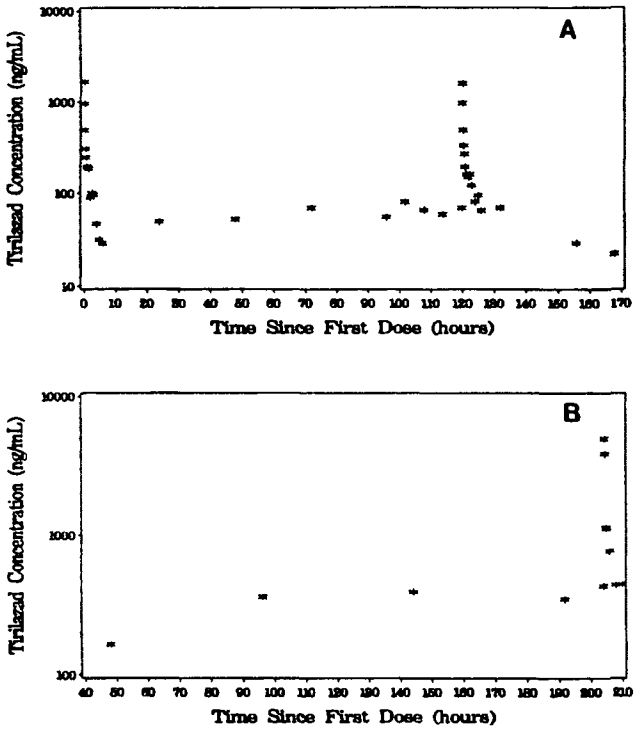

Fig. 1. Plasma tirilazad concentrations in A) a representative healthy volunteer receiving 2 mg/kg/day tirilazad mesylate and B) a representative SAH patient receiving 10 mg/kg/day tirilazad mesylate.

Table 3. Summary of Parameter Estimates and Standard Errors for the Basic Structural Model of Tirilazad

Parameter	Final parameter estimate		Magnitude of interindividual variability (%CV)	
	Mean	% SEM	Mean	% SEM
CL (L/hr)	27.5	2.8%	27.9%	17.9%
Vc (L)	5.65	16.6%	48.2%	38.3%
V_2 (L)	735	4.4%		
V_3 (L)	46.4	4.8%		
V_4 (L)	9.84	12.3%		
Q_{12} (L/hr)	10.7	3.1%		
Q_{13} (L/hr)	20.4	5.6%		
Q_{14} (L/hr)	28.1	4.5%		
Residual variability in normal volunteers (% CV)[a]	46.0%–24.8%	7.3%, 28.8%		
Residual variability in SAH volunteers (% CV)	50.0%	20.4%		

Note: Minimum value of the objective function = 70062.068.

[a] Residual variability in normal volunteers is estimated as a function of the predicted tirilazad concentration. The mean estimates are based on predicted concentrations ranging from 10–15,000 ng/mL.

error, and residual variability estimates for the basic structural model of tirilazad.

Patient Covariate Evaluations

The model incorporating a linear effect of weight on *CL* and *Vc* resulted in a statistically significant improvement in fit based on the addition of the two parameters (log likelihood difference = 218, $p < 0.01$). This model was then used as a basis for evaluating the effects of the three covariate subpopulations, i.e., normal volunteers not receiving phenytoin, normal volunteers receiving phenytoin, and patients with SAH receiving phenytoin, as well as a gender effect in each subgroup. The baseline subpopulation for comparison was male normal volunteers not receiving phenytoin.

The effect of each subpopulation was added to the models for *CL* and *Vc* incorporating a linear effect of weight. Then, using a backward elimination procedure, each effect associated with poor precision in the multivariable analysis (i.e., %SEM near 100%) was deleted from the model including all effects separately. In addition, subpopulations with well-estimated effects of similar magnitude (i.e., within 15%) for male and female groups were collapsed into one group by testing for the removal of the gender effect.

The effect of weight on *Vc*, the effect of gender on clearance in all subpopulations, and the effect of gender on *Vc* in normal volunteers receiving phenytoin were deleted from the model with no significant detriment in the fit to the data. All other effects were retained in the final model.

The final model describing *CL* includes a linear relationship with weight, a proportional shift for normal volunteers receiving phenytoin, and a proportional shift for SAH patients. No effect of gender on *CL* was found. The final model describing *Vc* includes a proportional shift for female, normal volunteers not receiving phenytoin, a proportional shift for normal volunteers receiving phenytoin, and separate proportional shifts for male and female patients with SAH. The models for *CL* and *Vc* are given below in Eqs. 8 and 9.

$$\tilde{CL}_j = [\Theta_{CL}^{int} + \Theta_{CL}^{wt} \cdot (WT_j - \overline{WT})] \cdot [1 + \Theta_{CL}^{nv.pht} \cdot (1 - SAH_j \cdot PHT_j)] \cdot [1 + \Theta_{CL}^{pt} \cdot SAH_j] \quad (8)$$

$$\tilde{Vc}_j = \Theta_{Vc} \cdot [1 + \Theta_{Vc}^{F.nv.nopht} \cdot SEXF_j \cdot (1 - SAH_j) \cdot (1 - PHT_j)] \cdot [1 + \Theta_{Vc}^{nv.pht} \cdot (1 - SAH_j) \cdot PHT_j] \cdot [1 + \Theta_{Vc}^{M.pt} \cdot SAH_j \cdot (1 - SEXF_j)] \cdot [1 + \Theta_{Vc}^{F.pt} \cdot SAH_j \cdot SEXF_j] \quad (9)$$

where:

$\Theta_{CL}^{nv.pht}$ = the mean increase or decrease in *CL* associated with the normal volunteers receiving phenytoin;

Θ_{CL}^{pt} = the mean increase or decrease in *CL* associated with SAH patients;

$\Theta_{Vc}^{F.nv.nopht}$ = the mean increase or decrease in *Vc* associated with female, normal volunteers, not receiving phenytoin;

$\Theta_{Vc}^{nv.pht}$ = the mean increase or decrease in *Vc* associated with normal volunteers receiving phenytoin;

$\Theta_{Vc}^{M.pt}$ = the mean increase or decrease in *Vc* associated with male SAH patients;

$\Theta_{Vc}^{F.pt}$ = the mean increase or decrease in *Vc* associated with female SAH patients;

Θ_{CL}^{int}, Θ_{CL}^{wt} = the intercept and slope of the relationship between *CL* and body weight for male and female normal volunteers not receiving phenytoin;

Θ_{Vc} = the volume of the central compartment for the reference population of male normal volunteers, not receiving concomitant phenytoin.

Table 4 summarizes the parameter, standard error and residual variability estimates for the final model. Figures 2 A and B show scatterplots of the measured versus predicted

Table 4. Summary of Parameter Estimates and Standard Errors for the Final Model, Including Patient Covariate Effects

Parameter	Final parameter estimate		Magnitude of interindividual variability (%CV)	
	Mean	% SEM	Population mean	% SEM
Θ_{CL}^{inl} (L/hr)	25.2	2.7%	24.7%	23.4%
Θ_{CL}^{wt} (L/hr/kg)	0.102	42.9%		
$\Theta_{CL}^{nv,pht}$	0.462	9.5%		
Θ_{CL}^{pt}	0.816	14.5%		
Θ_{Vc} (L)	4.56	13.5%	37.5%	23.0%
$\Theta_{Vc}^{F,nv,nopht}$	0.264	62.5%		
$\Theta_{Vc}^{nv,pht}$	−0.120	97.5%		
$\Theta_{Vc}^{M,pt}$	1.52	23.4%		
$\Theta_{Vc}^{F,pt}$	2.70	21.7%		
V_2 (L)	720	4.5%		
V_3 (L)	44.4	5.0%		
V_4 (L)	8.98	11.9%		
Q_{12} (L/hr)	10.4	3.3%		
Q_{13} (L/hr)	19.3	5.2%		
Q_{14} (L/hr)	25.1	5.8%		
Residual variability in normal volunteers (% CV)[a]	47.7%–21.5%	6.4%, 26.9%		
Residual variability in SAH volunteers (% CV)	66.3%	20.0%		

Note: Minimum value of the objective function = 68173.344.

[a] Residual variability in normal volunteers is estimated as a function of the predicted tirilazad concentration. The mean estimates are based on predicted concentrations ranging from 10–15,000 ng/mL.

concentrations and weighted residuals versus predicted concentrations from the final model, including patient covariate effects. Figure 3 depicts predicted versus measured plasma concentrations of tirilazad in each in the subpopulations of interest.

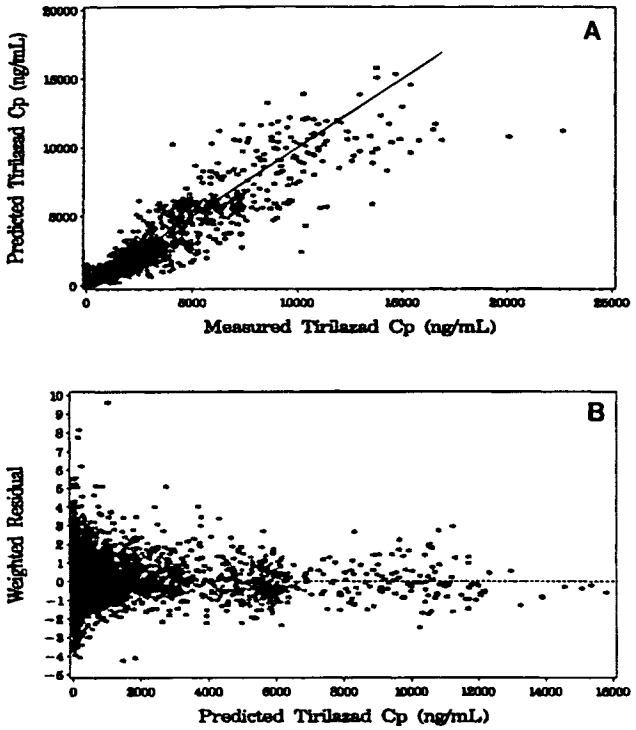

Fig. 2. Scatterplots of A) predicted versus measured tirilazad plasma concentrations, and B) weighted residuals versus predicted tirilazad concentrations. Results are based on the final model.

Two effects in the final model were estimated with relatively poor precision (i.e., %SEMs > 50%): the effect of female gender on the volume of the central compartment in the normal volunteers not receiving phenytoin and the effect of phenytoin on *Vc* in the normal volunteers. Both of these effects were highly negatively correlated with the estimate of *Vc* in male normal volunteers not receiving phenytoin. However, when these effects were tested for deletion from the multivariable model, they were found to be statistically significant. Throughout the analysis, the estimates of V_4 and Q_{14} were found to be positively correlated, indicating an inability of the model to independently estimate these parameters.

A marked decrease in the estimated magnitude of interindividual variability in both *CL* and *Vc* was noted when the effects of patient covariate subpopulations were incorporated into the model. Interindividual variability in *CL* decreased from 28% CV in the basic model to 25% CV in the final model. Likewise, the estimated interindividual variability in *Vc* decreased an even greater amount from 48% CV in the basic model to 38% CV in the final model. However, the estimated residual variability in SAH patients accounting for covariate subpopulation effects from 50% CV to 66% CV, while the residual variability in normal volunteers improved only slightly from a range of 46–25% CV to 48–22% CV over the range of predicted concentrations.

Based on the final model, normal volunteers receiving phenytoin were estimated to have a 46.2% increase in *CL* and a 12.0% decrease in *Vc*, as compared to normal volunteers not receiving phenytoin. With regard to *Vc*, however, female normal volunteers not receiving phenytoin were estimated to have a 26.4% increase as compared to males. In addition, SAH patients were predicted to have an 81.6% increase in *CL* and a 152% and 270% increase in *Vc* for male and female patients, respectively. A summmary of the effects is presented in Table 5.

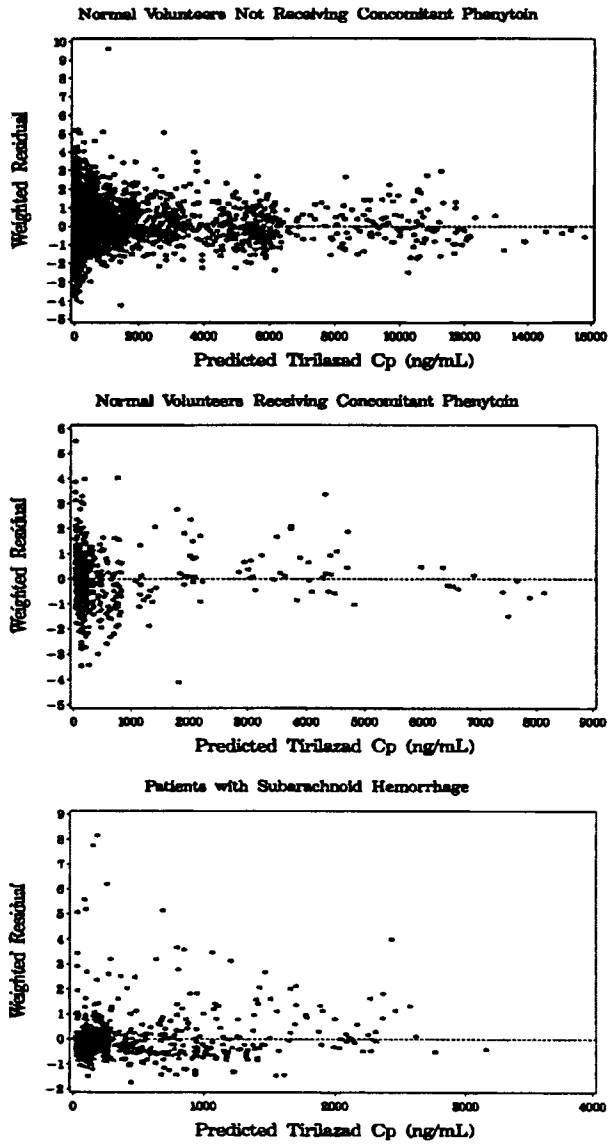

Fig. 3. Predicted versus measured tirilazad concentrations in three subpopulations of subjects. Results are based on the final model.

DISCUSSION

An effect of gender on tirilazad pharmacokinetics was identified in a study of the effect of age and gender on tirilazad pharmacokinetics (10). The results showed that tirilazad clearance was 40% higher in young women as compared to young men. This gender difference disappeared in elderly subjects, as tirilazad clearance decreased with age in women, but not men. At the time the results were published, the clinical significance of this finding was unknown.

In a study in Europe and Australasia, tirilazad was found to decrease mortality in men at a dose of 6 mg/kg/day, while no such effect was found in women (3). These results suggested that gender differences in tirilazad pharmacokinetics may have an effect on outcome in the treatment of SAH. A subsequent single dose study in healthy volunteers confirmed the difference between young females and males and suggested that age effects on tirilazad clearance were due to hormonal effects (11). However, the difference in tirilazad clearance between middle-aged males and females was approximately 20%. Multiple dose studies conducted in healthy male and female volunteers at about the same time indicated rather modest effects of gender on tirilazad pharmacokinetics (8,22). These results suggested that on multiple dosing in a middle-aged population, the group most at risk for experiencing a subarachnoid hemorrhage, any effect of gender on tirilazad pharmacokinetics would be minimal.

Likewise, the effect of phenytoin on tirilazad clearance in normal volunteers differed between two traditional pharmacokinetic trials. In study 39 (6), tirilazad clearance increased by approximately 50%; in study 52 (22), tirilazad clearance was doubled by the concomitant administration of phenytoin. Again, differences in study design (5 days versus 7 days of tirilazad administration) may have accounted for the divergence in results between studies.

To address these discrepancies, the present pooled analysis was conducted. After pooling the data from eleven studies, we still found the dataset lacking in information with regard to certain covariate effects, specifically the age and gender interaction effect. The youngest subjects in the dataset (<30 years of age) were 90% male and the oldest patients in the dataset (>60 years of age) were nearly 80% female. In addition, the young age group accounted for 50% of the male subjects and the older age group accounted for nearly 30% of the female subjects in the population. When this information was compounded with the fact that the patients, of whom all received concomitant phenytoin, tended to be female and the volunteers tended to be male, the population picture becomes even less well "mixed". Ideally, by pooling the data from so many studies, the resulting dataset would span over a wide range for continuous patient covariates, with equal or nearly equal representation of categorical covariates, e.g., gender, across these ranges and therefore, allow for an examination of the individual covariate effects as well as interaction effects. The correlation between age, weight and gender in the population studied herein precluded an examination of an age effect or an age*gender interaction effect. However, after eliminating patient subgroups with too small a representation for evaluation and interpretation, the database for analysis still permitted a sizable population of patients to explore the effects studied.

The variability in the model for the SAH patients in this analysis is greater than that of the healthy volunteers. This probably stems from variability introduced either through the incorrect recording of dosing times relative to sampling or from the fact that the early distribution phase of the concentration-time profile was missed in these patients. The latter problem could result in an underestimation of tirilazad AUC in this population and an overestimation of clearance. The increased residual variability for the patients with SAH in the final model compared with the basic model was initially thought to reflect the fact that the bulk of the concentrations measured in the patients were fit much better after incorporating patient covariate effects, but the most extreme measured concentration in a patient with SAH was actually more poorly predicted after accounting for covariate effects in the final model. However, when this concentration was removed from the dataset and the final model re-estimated, the magnitude of residual variability decreased by only a trivial amount (i.e., less than 1% CV) while all other parameter estimates were nearly identical to those obtained from the full dataset. Thus, this unusual concentration does not appear to exert an undue influence on the model fit,

Table 5. Summary of Significant Patient Covariate Effects on *CL* and *Vc* from this Population Analysis and Previous Studies

Patient covariate effect	Population PK analysis[a]		Previous traditional studies *CL*
	CL	*Vc*	
Base population = male normal volunteers, not receiving concomitant phenytoin	25.2 L/hr	4.56 L	23.3–32.2 L/hr (4) 22.3 L/hr (13) 26.1–55.2 L/hr (19) 34.9 L/hr (21) 28.1 L/hr (22)
Female normal volunteers, not receiving concomitant phenytoin	NSC[b]	26% ↑	38%–46% ↑ (12) 17%–60% ↑ (13)
Male normal volunteers receiving concomitant phenytoin	46% ↑	12% ↓	50% ↑ (8)
Female normal volunteers receiving concomitant phenytoin			
Male patients with SAH, receiving concomitant phenytoin	82% ↑	152% ↑	66.9–69.3 L/hr (15)
Female patients with SAH, receiving concomitant phenytoin		270% ↑	57.5–67.8 L/hr (15)

[a] All population analysis results are presented for subjects weighing 75.5 kg.
[b] NSC = No significant change from the base population.

and we are left with a substantially greater magnitude of residual variability for SAH patients as compared to normal volunteers.

The lack of a gender effect in the final model for tirilazad clearance is consistent with the results of multiple dose studies performed in healthy volunteers in the un-induced state. It is also consistent with the fact that as tirilazad clearance is induced by phenytoin, tirilazad clearance will approach hepatic blood flow. The large gender effects in traditional single dose studies may have been due to some degree to selection bias. The results of this population analysis thus indicate that gender is not a major factor affecting tirilazad clearance under multiple dose conditions. However, a large effect of gender was noted in *Vc* for the patients with subarachnoid hemorrhage and a smaller, less precisely estimated effect of gender in normal volunteers not receiving phenytoin was noted compared to male normal volunteers not receiving phenytoin.

Thus, the results of the present investigation suggest that any effect of gender on tirilazad pharmacokinetics is too modest to be solely responsible for the gender effect in response observed in SAH patients. Results of this and other investigations indicate that the major factor influencing tirilazad pharmacokinetics in SAH patients is concomitant phenytoin administration. Taken together, these results suggest that factors other than tirilazad pharmacokinetics (i.e., pharmacodynamic differences) may be responsible for mediating any gender difference in response to tirilazad in the treatment of SAH.

Overall, the results of the analysis indicate that the most important factors affecting tirilazad pharmacokinetics are the administration of phenytoin, an inducer of CYP3A, and SAH. The magnitude of the effect of concomitant phenytoin on clearance in normal volunteers is consistent with that seen in study 39, but was lower than that seen in study 52. Since tirilazad and phenytoin administration began at similar times in both patients and healthy volunteers in the studies included in this analysis, tirilazad clearance was probably time dependent throughout the study period. Induction of tirilazad clearance is evident within 48 hours of commencing phenytoin administration; deletion of early data for tirilazad was necessary to reasonably estimate the influence of phenytoin in these studies. It is possible that tirilazad clearance still varies enough over the remaining time period studied to result in an underestimation of phenytoin's effect on tirilazad clearance. Even if this effect is underestimated, the analysis still identified phenytoin coadministration as a covariate which substantially affects tirilazad clearance as well as volume of the central compartment. In the hospital setting, tirilazad and phenytoin dosing begin concomitantly, so the clinical significance of the interaction depends on whether plasma concentrations in the acute setting (prior to induction) or after several days of therapy are important for therapeutic efficacy.

The present analysis suggests an additional effect on both clearance and volume of the central compartment associated with SAH patients (Table 5). The differences between pharmacokinetic parameters estimated for SAH patients receiving concomitant phenytoin and normal volunteers receiving concomitant phenytoin observed in this analysis could be the result of a number of factors: (i) a high incidence of the use of triple-H therapy (hypervolemia, hypertension, hyperperfusion) in SAH patients in the U.S., (ii) an experimental artifact due to differing study and dosing durations or sampling schemes in the patient and volunteer studies included in this analysis, or (iii) other unknown metabolic differences between SAH patients and normal volunteers in this population.

In conclusion, the results of this analysis illustrate how data from a variety of studies conducted using traditional design and sampling strategies can be combined and analyzed using population pharmacokinetic analysis to gain an overall understanding of the factors affecting a drug's pharmacokinetics.

REFERENCES

1. J. M. Braughler, E. D. Hall, J. Jacobsen, J. M. McCall, and E. D. Means. The 21-aminosteroids: potent inhibitors of lipid peroxidation for the treatment of central nervous system trauma and ischemia. *Drugs Future* **14**:143–152 (1989).
2. N. F. Kassell, E. C. Haley, W. M. Alves, C. A. Hansen, B. Weir, and the participants. Phase two trial of tirilazad in aneurysmal subarachnoid hemorrhage. *Can. J. Neurol. Sci.* **20(Suppl 1)**: S29 (1993).
3. N. F. Kassell, E. C. Haley, C. Apperson-Hansen, W. M. Alves, and the participants. A randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage; a cooperative study in Europe/Australia/New Zealand. *J. Neurosurg.* **84**:221–228 (1996).

4. J. C. Fleishaker, L. K. Hulst, and G. R. Peters. Multiple dose tolerability and pharmacokinetics of tirilazad mesylate at doses of up to 10 mg/kg/day administered over 5-10 days in healthy volunteers. *Int. J. Clin. Pharmacol. Ther.* **32**:223–230 (1994).
5. L. C. Wienkers, R. C. Steenwyk, P. E. Sanders, and P. G. Pearson. Biotransformation of tirilazad in human: 1. Cytochrome P450 3A-mediated hydroxylation of tirilazad mesylate in human liver microsomes. *J. Pharmacol. Exp. Ther.* **277**:982–990 (1996).
6. J. C. Fleishaker, L. K. Hulst, and G. R. Peters. The effect of phenytoin on the pharmacokinetics of tirilazad mesylate in healthy male volunteers. *Clin Pharmacol Ther* **56**:389–397 (1994).
7. J. C. Fleishaker, L. K. Pearson, and G. R. Peters. Phenytoin causes a rapid increase in 6β-hydroxycortisol urinary excretion in humans—a putative measure of CYP3A induction. *J. Pharm. Sci.* **84**:292–294 (1995).
8. J. C. Fleishaker, L. K. Hulst-Pearson, and G. R. Peters. Gender does not affect the degree of induction of tirilazad clearance by phenobarbital. *Eur. J. Clin. Pharmacol.* **50**:139–145 (1996).
9. J. C. Fleishaker, P. G. Pearson, L. C. Wienkers, L. K. Pearson, and G. R. Peters. Biotransformation of tirilazad in human: 2. Effect of ketoconazole on tirilazad clearance and oral bioavailability. *J. Pharmacol. Exp. Ther.* **277**:991–998 (1996).
10. L. K. Hulst, J. C. Fleishaker, G. R. Peters, J. D. Harry, D. M. Wright, and P. Ward. Effect of age and gender on tirilazad pharmacokinetics in humans. *Clin. Pharmacol. Ther.* **55**:378–384 (1994).
11. J. C. Fleishaker, L. K. Hulst-Pearson, and G. R. Peters. Effect of gender and menopausal status on the pharmacokinetics of tirilazad mesylate in healthy subjects. *Am. J. Ther.* **2**:553–560 (1995).
12. J. C. Fleishaker, R. N. Straw, and C. J. Cross. Pharmacokinetics of tirilazad and U-89678, an active, reduced metabolite, following acute head trauma in adults. *J. Pharm. Sci.* **86**:434–437 (1997).
13. E. C. Haley, N. F. Kassell, C. Apperson-Hansen, M. H. Maile, W. M. Alves, and the participants. A randomized, double-blind, vehicle-controlled trial of tirilazad mesylate in patients with aneurysmal subarachnoid hemorrhage: a cooperative study in North America. *J. Neurosurg.* **86**:467–474 (1997).
14. J. C. Fleishaker, G. R. Peters, K. S. Cathcart, and R. C. Steenwyk. Evaluation of the pharmacokinetics and tolerability of tirilazad mesylate, a 21-aminosteroid free radical scavenger: II. Multiple-dose administration. *J. Clin. Pharmacol.* **33**:182–190 (1993).
15. J. C. Fleishaker, G. R. Peters, and K. S. Cathcart. Evaluation of the pharmacokinetics and tolerability of tirilazad mesylate, a 21-aminosteroid free radical scavenger: I. Single-dose administration. *J. Clin. Pharmacol.* **33**:175–181 (1993).
16. J. C. Fleishaker, L. K. Hulst, and G. R. Peters. Lack of a pharmacokinetic/ pharmacodynamic interaction between nimodipine and tirilazad mesylate in healthy volunteers. *J. Clin. Pharmacol.* **34**:837–841 (1994).
17. J. C. Fleishaker, L. K. Hulst, and G. R. Peters. Lack of pharmacokinetic interaction between cimetidine and tirilazad mesylate. *Pharm. Res.* **11**:341–344 (1994).
18. L. K. Hulst, J. C. Fleishaker, and G. R. Peters. Single dose proportionality of tirilazad mesylate pharmacokinetics in healthy male volunteers. *Pharm. Res.* **10(Suppl 1)**:S334 (1993).
19. L. K. Pearson, J. C. Fleishaker, and G. R. Peters. Comparison of the pharmacokinetics of tirilazad mesylate in healthy volunteers and stable subjects with mild liver cirrhosis. *Eur. J. Clin. Pharmacol.* **50**:299–304 (1996).
20. J. C. Fleishaker and G. R. Peters. Pharmacokinetics of tirilazad in healthy male subjects at doses above 6 mg/kg/day. *Int. J. Clin. Pharmacol. Ther.* **35**:28–32 (1997).
21. S. L. Beal and L. B. Sheiner, eds. NONMEM User's Guides, NONMEM Project Group, University of California at San Francisco, San Francisco, 1992.
22. J. C. Fleishaker, L. K. Pearson, and G. R. Peters. Induction of tirilazad clearance by phenytoin. *Biopharm. Drug. Disp.* **19**:91–96 (1998).