A Population Pharmacokinetic Model That Describes Multiple Peaks Due to Enterohepatic Recirculation of Ezetimibe

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ABSTRACT

Background: Ezetimibe, a selective inhibitor of intestinal cholesterol absorption, is in clinical development for the treatment of hypercholesterolemia. It is rapidly absorbed and glucuronidated in the intestine. The parent compound and its conjugated metabolite undergo enterohepatic recirculation, resulting in multiple peaks in the plasma concentration–time profile.

Objective: The purpose of this study was to develop a population pharmacokinetic (PPK) model for ezetimibe that incorporates enterohepatic recirculation.

Methods: A population compartment model incorporating input from the gallbladder, consistent with food intake, was developed to account for enterohepatic recirculation. The amount recycled was allowed to vary within a subject and between subjects, accommodating variability in bile secretion. The data used consisted of 90 profiles from healthy subjects who received single or multiple doses of ezetimibe 10 or 20 mg. Modeling was carried out using a nonlinear mixed-effect function in the S-PLUS® statistical program.

Results: The amount of ezetimibe recycled into the central compartment was estimated to be ~17% to 20% of the total amount absorbed, independent of the volume of distribution. The intersubject coefficient of variation was 46% to 80% in the absorption rate constant, 27% in the distribution phase, and ~50% in the volume of distribution.

Conclusions: PPK models adapted for enterohepatic recirculation allowed a formal assessment of the magnitude and frequency of the enterohepatic recirculation process, and the associated intersubject and intrasubject variability in healthy subjects. The PPK approach also helped to assess the correlation between the observed maximum or minimum (24 hours postdose) concentration with the model-based area under the curve, confirming the appropriateness of the former measures as a surrogate of drug exposure for a possible correlation with pharmacodynamics.

Key words: ezetimibe, cholesterol absorption inhibitor, population pharmacokinetics, enterohepatic recycling. (*Clin Ther.* 2001;23:871–885)

Accepted for publication April 11, 2001.

0149-2918/01/$19.00

INTRODUCTION

Ezetimibe (SCH 58235), a selective cholesterol absorption inhibitor, is in clinical development for the treatment of hypercholesterolemia. Ezetimibe has been shown to inhibit the absorption of dietary and biliary cholesterol in rats, rabbits, dogs, and cynomolgus and rhesus monkeys.[1–4] Ezetimibe is currently in phase III clinical trials, with results from 2 phase II trials in patients with primary hypercholesterolemia confirming that the drug significantly ($P < 0.05$) lowers low-density lipoprotein cholesterol in humans.[5,6] The molecular mechanism by which this compound inhibits cholesterol absorption is currently being elucidated.

Clinical studies completed to date involving >1500 healthy subjects and hypercholesterolemic patients demonstrated that ezetimibe is well tolerated.[5–9] The overall incidence of adverse events with ezetimibe was similar to that associated with placebo. The most commonly reported adverse effects were generally mild, transient, and nonspecific and included headache and gastrointestinal complaints.[5,6]

Ezetimibe is excreted into the bile after undergoing extensive glucuronidation in the intestine to a phenolic glucuronide, SCH 60663.[10] It is possible that ezetimibe is repeatedly delivered back to the site of action, the lumen of the intestinal tract, via enterohepatic recirculation (EHC) after undergoing reabsorption in the ileum. This, in turn, has the potential to enhance the residence time of the compound in the lumen of the intestinal tract, thereby potentiating its cholesterol-lowering activity.[10]

EHC has been shown to contribute to multiple peaks in the plasma concentration–time profile of various compounds.[11,12] Because conventional pharmacokinetic models are unsuitable for such data,[13] these models have been modified to incorporate additional input into the intestine from EHC.[12,14,15] In this study, we followed a similar approach but adapted a population pharmacokinetic (PPK) model and applied the model to pooled data from clinical trials in healthy volunteers receiving single or multiple doses of ezetimibe. Since the glucuronide (~90% of the total) and the parent compound both exhibit multiple peaks and exhibit cholesterol-lowering activity, the total ezetimibe concentrations (glucuronide + parent) were modeled.

Based on the model parameters, individual pharmacokinetic parameters were also derived and correlated with the observed maximum and minimum plasma concentrations to establish the appropriateness of the latter parameters as surrogate measures of drug exposure for possible correlations with pharmacodynamic responses.

METHODS

Study Population and Drug Administration

Data from 5 phase I clinical studies[16–20] were pooled and used in this investigation. These were well-supervised, inpatient pharmacokinetic studies in which meals were standardized with regard to their timing relative to ezetimibe dosing.

Two merged datasets were generated: dataset A contained full profiles from 54 subjects after a single dose of ezetimibe 20 mg. Dataset B contained full profiles on Day 1 (single dose) and Day 10 (multiple dose) from 36 subjects receiving either 10 or 20 mg ezetimibe. All subjects were young healthy males or females who

had a 10-hour fast before the first dose and who were not allowed to eat until at least 2 or 4 hours postdose. Water intake was allowed during the fasting period.

Ethics

Before the initiation of each study, the protocols and statements of informed consent were reviewed and approved by an institutional review board. The studies were conducted in accordance with Good Clinical Practices. Procedures were in compliance with the US Code of Federal Regulations (21 CFR Parts 50 and 56), with the World Medical Association Declaration of Helsinki concerning informed consents and the protection of rights of human subjects, and with the internal standard operating procedures of Schering-Plough Research Institute, Kenilworth, New Jersey. Written informed consent was obtained from each volunteer before performing any study-related activities.

Pharmacokinetics

Blood samples were collected for the determination of total ezetimibe concentrations in plasma (total ezetimibe is the sum of unchanged [unconjugated] ezetimibe and ezetimibe–glucuronide). For single-dose administration, the combined sampling times were at 0 hour (predose) and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, and 48 hours postdose. For multiple-dose administration, the combined sampling times were at 0 hour (predose) and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, and 24 hours postdose. All blood samples were centrifuged within 30 minutes after collection for 10 to 15 minutes at ~1000 to 1500*g* and 4°C. The plasma was separated, transferred into a cryogenic tube, and stored frozen at –20°C or below until analyzed.

Plasma samples were assayed for ezetimibe and total ezetimibe concentrations using validated liquid chromatography/tandem mass spectrometry methods at Phoenix International Life Sciences, St.-Laurent, Quebec, Canada. The analytic methods had lower limits of quantitation of 20 pg/mL and 0.250 ng/mL, and calibration curve ranges of 20.0 to 20,000 pg/mL and 0.250 to 250 ng/mL for unchanged and total ezetimibe, respectively. The coefficients of variation for the quality-control samples ranged from 5.2% to 8.7% between runs and from 3.2% to 8.6% within runs.

For the analysis of unchanged ezetimibe, 100 μL of internal standard working solution ($^{13}C_6$-SCH 58235, 150 ng/mL in water) and 1.0 mL water were added to a 1-mL aliquot of each sample before extraction with 8.0 mL of 1-chlorobutane. For the determination of total ezetimibe, 100 μL of internal standard working solution (250 ng/mL), 500 μL of sodium acetate (0.5M, pH 5.0) and 50 μL of β-glucuronidase (100,000 units/mL) were added to each 200-μL aliquot of plasma. The samples were then incubated at 50°C for 60 minutes and 500 μL of sodium borate solution (0.1M) was added. The samples were then extracted with 8.0 mL of 1-chlorobutane. Each tube was then shaken for 15 minutes at room temperature and centrifuged. After centrifugation, the samples for the determination of ezetimibe and total ezetimibe were processed similarly. The organic layer was removed and evaporated using a Turbovap® (Zymark Corporation, Hopkington, Massachusetts) or a SpeedVac® apparatus (Thermo Savant, Holbrook, New York), reconstituted in 500 μL of methanol, and

again evaporated to dryness. The residues were then reconstituted in 50 μL of 20:80 (v/v) water in methanol and analyzed using a Waters Alliance 2690 high-performance liquid chromatograph (Waters Corporation, Milford, Massachusetts) equipped with a Sciex API III mass spectrometer (Perkin-Elmer, Norwalk, Connecticut). We used a 7.5 cm × 4.6 mm Zorbax SB-C18 chromatographic column with 3.5-μm particle size (Chromatographic Specialties, Brockville, Ontario, Canada) and an isocratic mobile phase composed of 80% methanol:20% 0.025 mol/L ammonium acetate (1.0 mL/min flow rate).

Mass spectrometric detection was performed in the positive-ion mode. The m/z transitions monitored for SCH 58235 were 392.0 to 133.4, and those for the internal standard were 398.4 to 139.4. Plasma concentrations of ezetimibe–glucuronide, reported as ezetimibe equivalents, were calculated for each plasma sample by subtracting the unchanged ezetimibe concentration from the corresponding total ezetimibe concentration.

Pharmacokinetic Modeling

The pharmacokinetic profile of ezetimibe was characterized using a 2-compartment model[21] incorporating random effects.[22] In the absence of EHC, plasma concentrations were modeled as follows:

$$C_j(t) = C_{pred,j}(t) + \epsilon_j(t) \qquad (1)$$

where $C_j(t)$ and $C_{pred,j}(t)$ are the measured and predicted concentration for the *j*th subject at time t, respectively. The intrasubject variability in concentration, including measurement and assay error $\epsilon_j(t)$, was assumed to have a normal distribution (with mean 0 and variance σ^2_ϵ). The intersubject variability in the pharmacokinetic parameters was modeled as a proportionality term. For example, volume of distribution in the central compartment (V) for the *j*th subject was defined as:

$$V_j = V \cdot \exp(\eta_{j,V}) \qquad (2)$$

where V, as a fixed effect, is the population estimate and $\exp(\eta_{j,V})$ is the deviation in V for the *j*th subject. The random effect $\eta_{j,V}$ was assumed to follow a normal distribution (with mean 0 and variance σ^2_V). The intersubject coefficient of variation (CV) in V is thus approximated by the estimate of σ_V. Subject characteristics, such as age, body weight, and sex, were not explored since ~80% of the subjects were young, healthy males. The η values for the different parameters were assumed to be independent of each other. Since V and the bioavailability (F) cannot be estimated independently, F was fixed at 1 to allow for estimation of the relative volume V/F.

EHC was incorporated in the PPK model as a secondary input into the intestinal tract. The gallbladder emptying was postulated to be at mealtime, that is, at 4, 8, 12, and 24 hours postdose for single-dose administration and at 0, 4, 8, and 12 hours postdose for multiple-dose administration. It is well known that food stimulates bile secretion[23] and the resulting biliary secretion was assumed to deliver a fraction of the amount absorbed into the intestinal tract.

With EHC, a fraction δ_k is added back to the systemic circulation at time τ_k, where k = 1, 2, ..., n, and n is the number of such events. Equation 1 is thus modified as:

$$C_j(t) = C_{pred,j}(t) + \delta_1 \cdot C_{pred,j}(t - \tau_1) + \delta_2 \cdot C_{pred,j}(t - \tau_2) + \cdots + \delta_n \cdot C_{pred,j}(t - \tau_n) + \epsilon_j(t) \qquad (3)$$

where $C_{pred,j}(t - \tau_k)$ is 0 for $t \leq \tau_k$. Equation 3 is thus similar to that of a multiple-dose profile at times τ_k using the principle of superpositioning.

Random effects to account for variability in δ_k and in τ_k were introduced in the model as described in equation 2.

The PPK model was first fit to dataset A and was reevaluated with dataset B as a model-validation step. Distribution assumptions were examined for validity using normality tests and graphical methods. Model goodness-of-fit was assessed using analysis of residuals. PPK modeling was performed using nonlinear mixed-effects (NLME) models, a function of the statistical package S-PLUS®[24]; additional details about the NLME models can be found elsewhere.[25–27]

RESULTS

Pharmacokinetics

Pharmacokinetic data in dataset A included profiles from 54 subjects with 822 plasma samples. Dataset B included 2 profiles from each of 36 subjects, 1 profile after a single-dose administration and another after multiple-dose administration. There were 492 plasma samples from each of the single-dose and multiple-dose administrations in dataset B. A subset of the subjects' profiles from datasets A and B are illustrated in Figures 1 and 2, respectively. In dataset A, the median maximum concentration (C_{max}) was 85 ng/mL obtained at a median time (T_{max}) of 1 hour after dosing. In dataset B, the values for

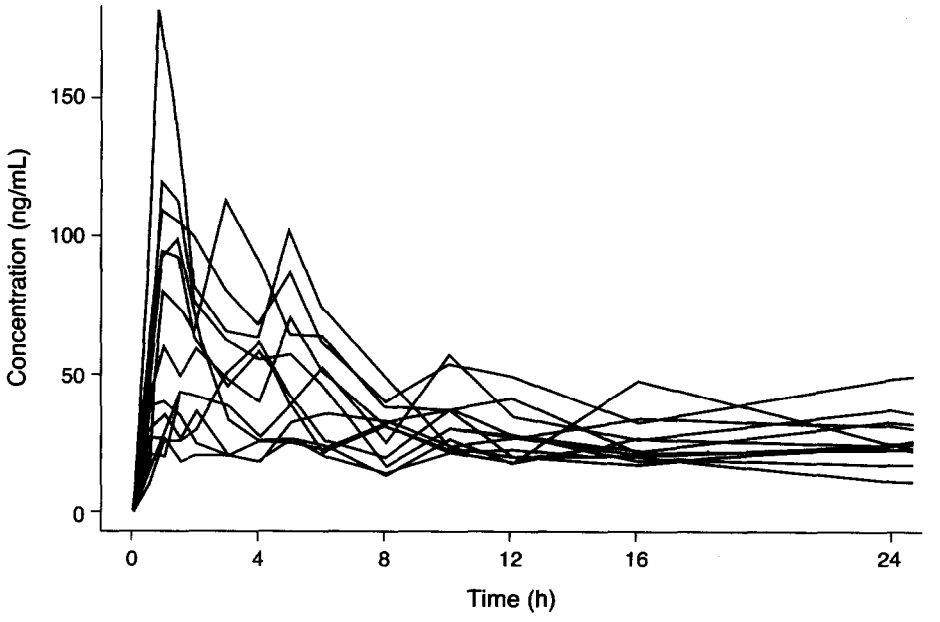

Figure 1. Plasma ezetimibe concentration–time profiles after a single-dose administration of ezetimibe.

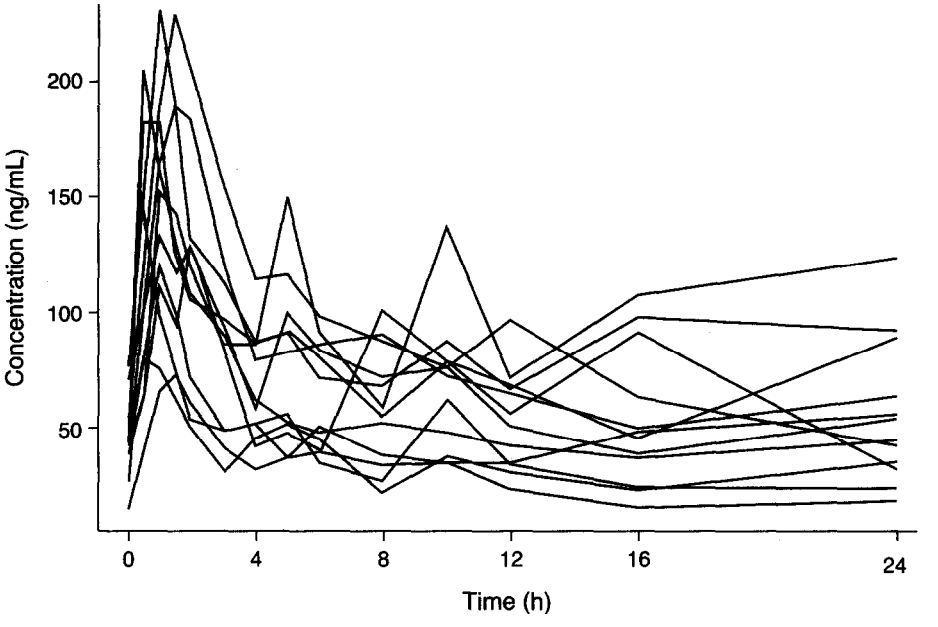

Figure 2. Plasma ezetimibe concentration–time profiles after multiple-dose administration of ezetimibe.

C_{max} and T_{max} after multiple-dose administration were 122 ng/mL and 1 hour. Concentration values from pairs of time points were compared to describe the frequency and magnitude of EHC. For notational convenience, we let C_u denote the concentration at hour u from dosing and the ratio $R_{u,v} = (C_u - C_v)/C_v$, $u > v$. Then, for example, $R_{5,4}$ would represent the change in concentration at 5 hours compared to that at 4 hours. $R_{5,4}$ was plotted against C_4 (the concentration at 4 hours postdose) for the 54 subjects in dataset A (Figure 3). The smoothing line through the points demonstrates that the increase is highest for subjects with the lowest 4-hour concentrations. Subjects with C_4 values higher than the median, indicated by the vertical line at 36 ng/mL, had, on average, similar C_5 values, suggesting equilibrium between input primarily due to EHC and output due to elimination. A similar observation was made for $R_{10,8}$ (Figure 4) and for $R_{24,16}$ (not shown), suggesting that EHC is still present at 24 hours. In contrast, the majority of subjects had $R_{4,3}$ values <0, that is, a decline in concentration at 4 hours (Figure 5), indicating negligible EHC at this time postdose.

Population Pharmacokinetics

Parameter estimates for the selected model, a 2-compartment model with first-order absorption and with elimination from the central compartment, are shown in Tables I and II for datasets A and B, respectively. The model fits the data well,

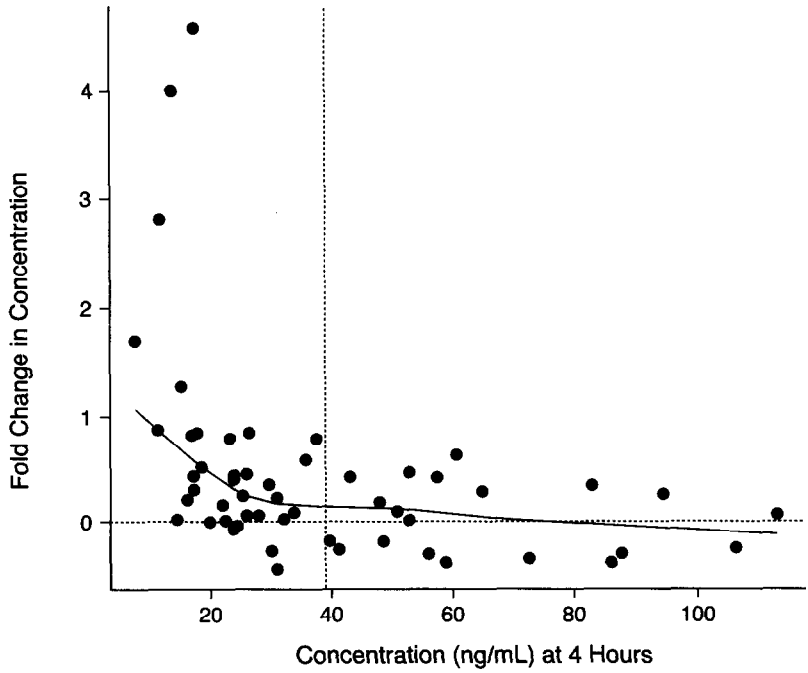

Figure 3. Fold change in 5-hour relative to 4-hour ezetimibe plasma concentration plotted against ezetimibe concentration at 4 hours.

as indicated by the observed versus fitted residuals and random-effect plots. Figure 6 is a plot of the individual plasma ezetimibe concentrations in dataset A, the observed median concentration, and the model-estimated median. In Figures 7 and 8, plots of observed versus fitted and residual versus fitted concentration, respectively, are shown for dataset B. A plot of observed and fitted concentration versus time for 2 subjects with low and high maximum concentrations is shown in Figure 9. The population absorption half-life was 0.5 to 0.8 hours. C_{max} was estimated to occur at 1.3 and 1.4 hours, and to reach 74 ng/mL and 131 ng/mL after single-dose and multiple-dose administration, respectively. The estimated terminal elimination half-life was 30 hours. There was high intersubject variability in absorption rate (Ka, 46%–80%) and in volume (45%–49%). The terminal slope, β, and the transfer rate constant from the peripheral compartment to the central compartment (K_{21}) did not vary significantly between subjects; therefore, their variance terms were set to 0. The amount reabsorbed was found to be similar at different meal times (ie, $\delta_1 = \delta_2 = ... = \delta_n$). There were no significant deviations from the postulated meal times 4, 8, 12, and 24 hours after single-dose administration and 0, 4, 8, and 12 hours after multiple-dose administration. No significant differences were found between subjects; thus a common δ (SE) value was

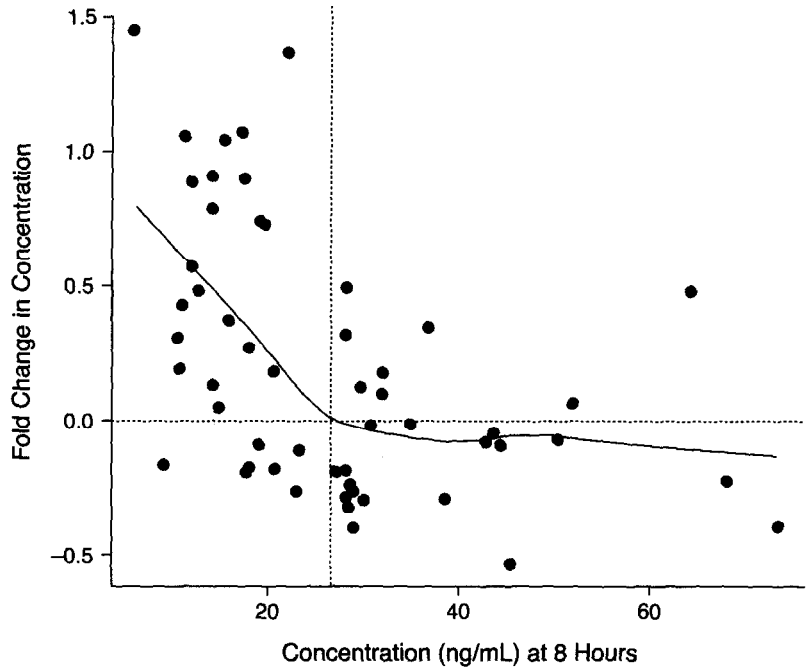

Figure 4. Fold change in 10-hour relative to 8-hour ezetimibe plasma concentration plotted against ezetimibe concentration at 8 hours.

estimated—0.2 (0.03) for dataset A and 0.17 (0.03) for dataset B.

DISCUSSION

A previous study[10] has shown that ezetimibe in the animal model is extensively glucuronidated to a phenolic glucuronide (SCH 60663) in the intestinal enterocyte and is delivered back to the lumen of the intestine via bile. It was also suggested in the same study that the phenolic glucuronide can undergo enzymatic hydrolysis in the intestinal lumen, delivering the parent compound back into the intestinal lumen where it is reabsorbed, a process known as enterohepatic recirculation. This enzymatic hydrolysis of glucuronide to the parent drug appears to be complete since the glucuronide is not absorbed[10] and is not detected in the feces.[28]

Clinical studies with ezetimibe have shown multiple peaks in the plasma concentration–time profiles (within a dosing interval), which are similarly attributed to EHC. In this paper, we evaluated the contribution of EHC to plasma concentrations using pooled data from phase I studies employing a population pharmacokinetic model. To obtain a homogeneous population, only young, healthy, fasting subjects receiving 10 or 20 mg ezetimibe as a single agent were included in this analysis. These data were obtained from 5 different studies meeting this criterion. The pharmacokinetic model adapted for EHC was

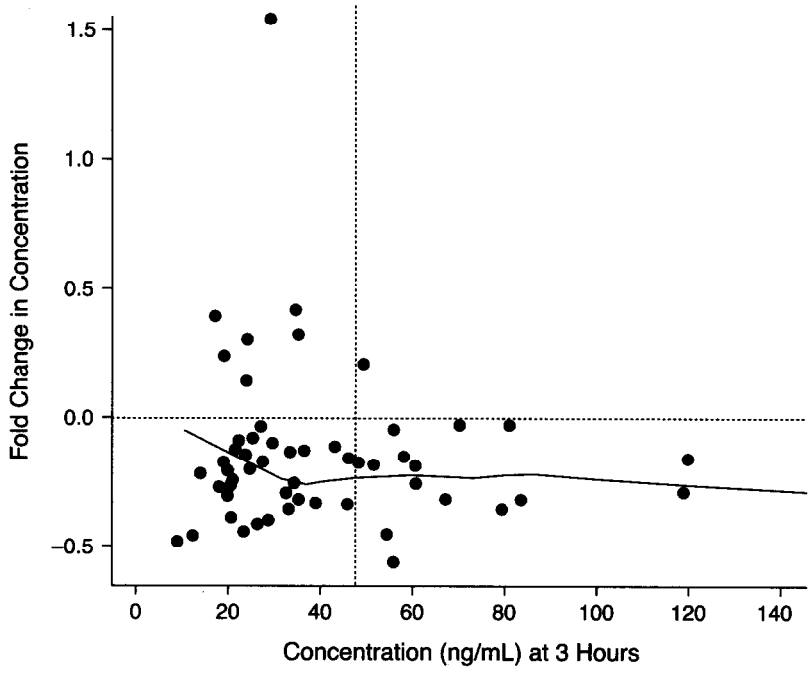

Figure 5. Fold change in 4-hour relative to 3-hour ezetimibe plasma concentration plotted against ezetimibe concentration at 3 hours.

Table I. Ezetimibe parameter estimates from the population pharmacokinetic model (N = 54): Single-dose administration.

Parameter	Estimate	Standard Error of Estimate	Intersubject Coefficient of Variation (%)
Ka, hr^{-1}	0.82	0.14	80
α, hr^{-1}	0.79	0.11	27
β, hr^{-1}	0.023	0.006	NS
K_{21}, hr^{-1}	0.087	0.016	NS
V/F, L	107.5	15.3	49
δ	0.20	0.03	NS
σ_ϵ, ng/mL	13.9		

Ka = absorption rate; α = micro rate constant of the distribution phase; β = terminal slope; NS = not significant; K_{21} = transfer rate constant from the peripheral to the central compartment; V/F = relative volume; δ = fraction reabsorbed due to enterohepatic recirculation; σ_ϵ = intrasubject standard deviation.

Table II. Ezetimibe parameter estimates from the population pharmacokinetic model (N = 36): Single- and multiple-dose administration.

Parameter	Estimate	Standard Error of Estimate	Intersubject Coefficient of Variation (%)
Ka, hr^{-1}	1.57	0.25	46
α, hr^{-1}	0.67	0.10	27
β, hr^{-1}	0.025	0.003	NS
K_{21}, hr^{-1}	0.095	0.016	NS
V/F, L	105.3	13.3	45
δ	0.17	0.03	NS
σ_ϵ, ng/mL	21.1		

Ka = absorption rate; α = micro rate constant of the distribution phase; β = terminal slope; NS = not significant; K_{21} = transfer rate constant from the peripheral to the central compartment; V/F = relative volume; δ = fraction reabsorbed due to enterohepatic recirculation; σ_ϵ = intrasubject standard deviation.

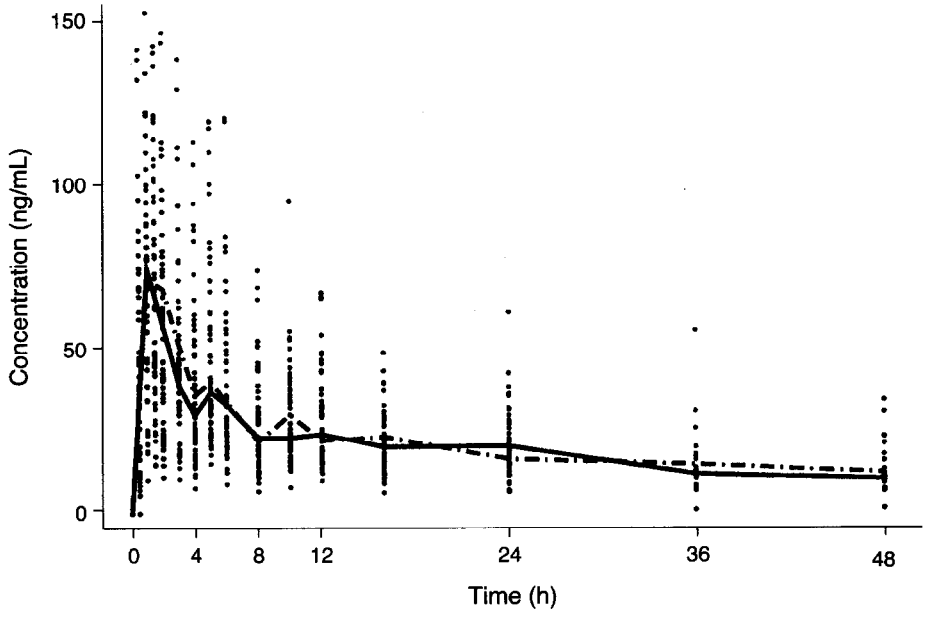

Figure 6. Individual ezetimibe concentration values (dots), observed median concentration values (broken line), and model-estimated median concentration values (solid line) for dataset A.

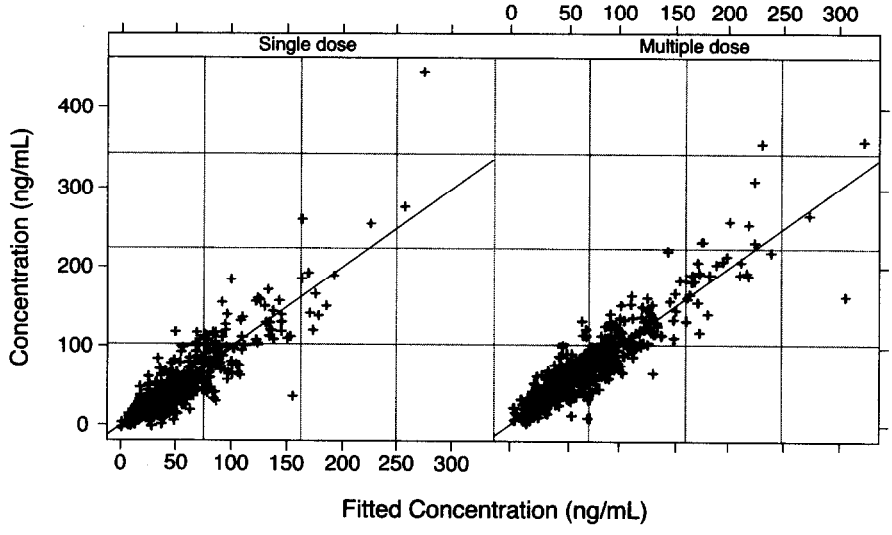

Figure 7. Observed versus fitted ezetimibe plasma concentration in dataset B. Left panel, single dose; right panel, multiple dose.

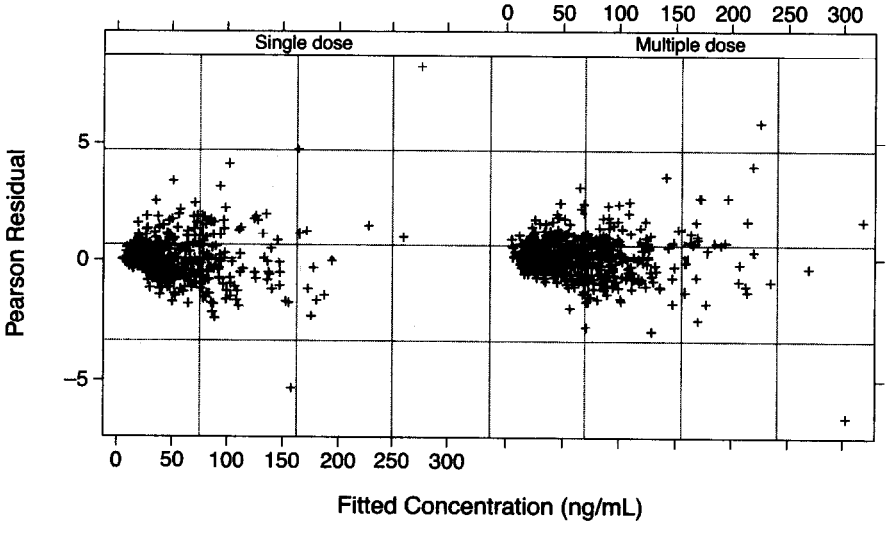

Figure 8. Pearson residual versus fitted ezetimibe plasma concentration in dataset B. The line is the line of unity when residual = 0. Left panel, single dose; right panel, multiple dose.

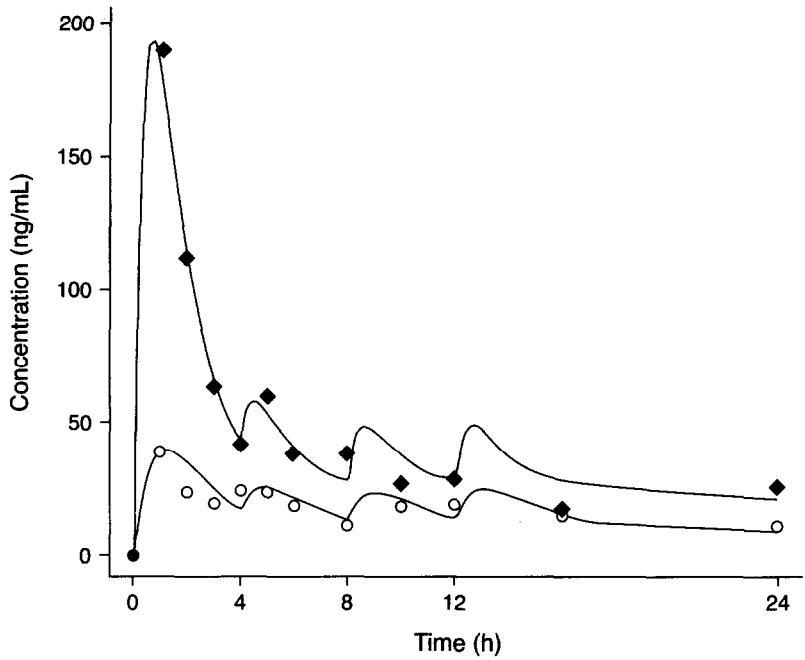

Figure 9. Observed (open circles or solid diamonds) and fitted (solid lines) plasma ezetimibe concentrations versus time for 2 subjects, 1 with low (open circles) and 1 with high (solid diamonds) maximum concentration values.

a 2-compartment open model. EHC was modeled as an additional input into the systemic circulation, amounting to a percentage (δ) of the absorbed dose. Since EHC was considered to occur at or near meal times (eg, 4, 8, and 12 hours after drug administration), the amount and time of EHC were initially allowed to vary within and between subjects. No significant improvement in the fit was achieved with variable times or variable amount reabsorbed.

Furthermore, no significant differences were found between single-dose and multiple-dose administration in δ or in other pharmacokinetic parameters. These results suggest that the addition of a single parameter, δ, representing a fixed amount in all subjects, does in fact explain most of the multiple peaks. Additional parameters or random effects were found to reduce residual errors slightly, without affecting the structural parameters. Because of the minor gain in model fit, and for reasons of parsimony, these effects were dropped from the final model. After single-dose administration, δ was estimated to be 20% compared with 17% at steady state. This amount was found not to correlate with volume of distribution, demonstrating that subjects with initial high peaks do not necessarily achieve similarly high peaks due to EHC. The results from PPK fittings suggested enterohepatic recycling every 4 hours consistent with physiology, that is, gallbladder vol-

ume remains almost constant during the fasting condition and food intake triggers the emptying of the gallbladder.[23] The amount of drug released with the bile into the lumen of the intestinal tract as a result of gallbladder emptying is thus a secondary input. This amount is absorbed in a manner similar to the administered dose and at a similar absorption rate. As evident in Figures 2 and 3, not all subjects had equal EHC at the same time. This was probably due to the differences in meal times relative to the ezetimibe dose across studies (ie, 2 hours vs 4 hours postdose) as well as the variability associated with the triggering of gallbladder emptying. However, in the majority of subjects, an increase followed by a decrease in concentration was observed approximately every 4 hours, 3 to 4 times postdose (Figures 3–5).

The population estimates suggested that ezetimibe is rapidly absorbed. Although the estimate from dataset A was lower than that from dataset B, this difference was not attributed to the mode of administration. Rather, it is more likely due to intersubject and interstudy variability, which was verified by further modeling with subsets of the data. The remaining rate constant and volume of distribution were similar in the 2 datasets. The model-based area under the curve (AUC) derived from equation 3 (AUC1) gave a median estimate of 1554 (CV = 43%). Using $\delta = 0$ in this equation, that is, assuming no EHC, gave a median estimate (AUC2) of 878 (CV = 65%). The ratio AUC2/AUC1 correlated significantly (declined exponentially) with AUC1, with the observed concentration at 24 hours, and with C_{max}, demonstrating that the percentage contribution of EHC was highest in subjects with the lowest drug exposure. Because of enterohepatic recycling and the resulting reabsorption, the model-estimated mean AUC value was increased by about 86% (33% SD) compared to the value in the absence of EHC. Thus, the phenomenon of EHC significantly increased the residence time of ezetimibe at the site of action, which may result in a beneficial cholesterol-lowering effect.

The model-estimated median C_{max}, C_{min} (concentration at 24 hours), and T_{max} were 74 ng/mL, 16 ng/mL, and 1.3 hours, respectively. These values were comparable to the corresponding observed values (74 ng/mL, 19 ng/mL, and 1 hour, respectively). The same parameters estimated by the model at steady state were 131 ng/mL, 44 ng/mL, and 1 hour, indicating that although C_{max} at steady state increased only ~2-fold, C_{min} increased ~3-fold. The likely explanation for the larger increase in C_{min} than in C_{max} is the contribution of EHC in the later phases of the ezetimibe plasma concentration profile. The model-estimated AUC also correlated with both observed C_{min} and C_{max} ($P < 0.001$). The data used in this investigation are obtained from healthy subjects under strict conditions. However, the main results regarding the amount of drug recycled are likely to be similar in the target patient population, since hypercholesterolemia and other concomitant disorders are not expected to significantly alter enterohepatic recycling.

CONCLUSIONS

A PPK model for ezetimibe was developed that included additional input due to enterohepatic recycling. The model provided a reasonable approximation of individual profiles for most subjects after single-dose or multiple-dose administration, enabling a formal description of the pharmacoki-

netic properties of ezetimibe in the presence of EHC, including rate parameters and intersubject variability.

ACKNOWLEDGMENTS

We thank Leon Aarons, Kenneth Bauer, and Yali Zhu for their useful comments during the investigation.

REFERENCES

1. Salisbury BG, Davis HR, Burrier RE, et al. Hypercholesterolemic activity of a novel inhibitor of cholesterol absorption, SCH 48461. *Atherosclerosis.* 1995;115: 45–63.

2. Sybertz EJ, Davis HR, Van Heek M, et al. SCH 48461, a novel inhibitor of cholesterol absorption. In: Woodford FP, Davignon J, Sniderman A, eds. *Atherosclerosis X:* Proceedings of the 10th International Symposium on Atherosclerosis, Montreal, October 9–14, 1994. New York: Elsevier; 1995:311–315.

3. Van Heek M, France CF, Compton DS, et al. In vivo metabolism-based discovery of a potent cholesterol absorption inhibitor, SCH58235, in the rat and rhesus monkey through the identification of the active metabolites of SCH48461. *J Pharmacol Exp Ther.* 1987;283:157–163.

4. Rosenblum SB, Huynh T, Afonso A, et al. Discovery of 1-(4-fluorophenyl)-(3*R*)-[3-(4-fluorophenyl)-(3*S*)-hydroxypropyl]-(4*S*)-(4-hydroxyphenyl)-2-azetidinone (SCH 58235): A designed, potent, orally active inhibitor of cholesterol absorption. *J Med Chem.* 1998;41:973–980.

5. Lipka LJ, Lebeat AP, Veltri EP, et al. Reduction of LDL-cholesterol and elevation of HDL-cholesterol in subjects with primary hypercholesterolemia by ezetimibe (SCH 58235): Pooled analysis of two phase II studies. *J Am Coll Cardiol.* 2000; 35(Suppl A):257A. Abstract 1046-21.

6. Bays H, Drehobl M, Rosenblatt S, et al. Low-density lipoprotein cholesterol reduction by SCH 58235 (ezetimibe), a novel inhibitor of cholesterol absorption, in 243 hypercholesterolemic subjects: Results of a dose-response study. *Atherosclerosis.* 2000;151:133. Abstract TuP15:W16.

7. Castañer RM, Sorbera LA, Castañer J. Ezetimibe. Hypolipidemic, cholesterol absorption inhibitor. *Drugs Future.* 2000;25: 679–685.

8. Brown WV. Novel approaches to lipid lowering: What is on the horizon? *Am J Cardiol.* 2000;87(Suppl):23B–27B.

9. Thompson GR, Naoumova RP. Novel lipid-regulating drugs. *Expert Opin Invest Drugs.* 2000;9:2619–2628.

10. Van Heek M, Farley C, Compton DS, et al. Comparison of the activity and disposition of the novel cholesterol absorption inhibitor, SCH58235, and its glucuronide, SCH60663. *Br J Pharmacol.* 2000;129: 1748–1754.

11. Pedersen PV, Miller R. Pharmacokinetics and bioavailability of cimetidine in humans. *J Pharm Sci.* 1980;69:394–398.

12. Shepard TA, Reuning RH, Aarons LJ. Estimation of area under the curve for drugs subject to enterohepatic cycling. *J Pharmacokinet Biopharm.* 1985;13:589–608.

13. Krishna G, Barr WH, Mikulecky DC. Use of SPICE, an electrical circuit analysis program, to simulate the occurrence of double peaks and evaluate critical factors in plasma concentration-time profiles after oral drug administration. *Pharm Res.* 1992;9:S332.

14. Plusquellec Y, Houin G. Drug recirculation model with multiple cycles occurring at unequal time intervals. *J Biomed Eng.* 1992;14:521–526.

15. Plusquellec Y, Arnaud R, Saivin S, et al. Enterohepatic recirculation of the new antihypertensive drug UP 269-6 in humans. *Arzneim-Forsch Drug Res.* 1998;48:138–144.

16. Punwani N, Pai S, Bach C, et al. Effect of food on oral bioavailability of SCH 58235 in healthy male volunteers. *Am Assoc Pharm Sci Suppl.* 1998;1:S486. Abstract 3426.

17. Zhu Y, Statkevich P, Kosoglou T, et al. The effect of gender on the pharmacokinetics of SCH 58235, a cholesterol absorption inhibitor. *Am Assoc Pharm Sci Suppl.* 1999;1:S24. Abstract.

18. Zhu Y, Statkevich P, Kosoglou T, et al. Effect of age on the pharmacokinetics of ezetimibe. *Am Assoc Pharm Sci Suppl.* 2000;2:2082. Abstract.

19. SCH 58235: Rising, single-dose safety and tolerance study in healthy male volunteers (study report for protocol no. I96-088). Kenilworth, NJ: Schering-Plough Research Institute; 1997. Clinical document 97205350.

20. SCH 58235: Bioavailability of single oral doses of two prototype tablet formulations and the reference capsule formulation of SCH 58235 in normal male volunteers; a four way crossover study (study report for protocol no. C97-221). Kenilworth, NJ: Schering-Plough Research Institute; 1998. Clinical document 98177125.

21. Gibaldi M, Perrier D. *Pharmacokinetics.* 2nd ed. New York: Marcel Dekker; 1982: 81–84.

22. Scheiner LB, Rosenberg B, Marathe VV. Estimation of population characteristics of pharmacokinetic parameters for routine clinical data. *J Pharmacokinet Biopharm.* 1977;5:445–479.

23. Fuchs WS, von Nieciecki A, Molz KH, et al. The effect of bile secretion on the pharmacokinetics of a theophylline sustained-release preparation. *Arzneim-Forsch Drug Res.* 1998;48:597–604.

24. *S-PLUS 2000 Guide to Statistics.* Vol 1. Seattle: MathSoft Data Analysis Products Division.

25. Lindstrom ML, Bates DM. Nonlinear mixed effects models for repeated measures data. *Biometrics.* 1990;46:673–687.

26. Davidian M, Giltinan DM. *Nonlinear Models for Repeated Measurement Data.* London: Chapman & Hall; 1995.

27. Pinheiro JC, Bates DM. *Mixed-Effects Models in S and S-plus.* New York: Springer-Verlag; 2000.

28. Iannucci RM, Kaczynski EJ, Achanfuo-Yeboah J, et al. Metabolism of SCH 58235 in the human, rat and dog. *Proceedings of the 47th ASMS Conference on Mass Spectrometry and Allied Topics.* 1999;364–365.

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