EXAMINING SOURCES OF INTERINDIVIDUAL PHARMACOKINETIC VARIABILITY BY NONLINEAR MIXED EFFECTS MODELING

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Abstract

The application of the nonlinear mixed effects modeling to pharmacokinetics can maximize the goals of drug administration from the time a drug is first administered in human during the initial phases of development to the routine patient care. Nonlinear mixed effects modeling has a pivotal role in population pharmacokinetics, which is especially valuable since it targets the patient group that will eventually receive the drug of interest. It is applicable in sparse data situations, quantifies pharmacokinetic variability at several levels and aims to explain the sources of variability. When applied to direct patient care, the purpose of nonlinear mixed effects modeling is to provide quantitative and semi-quantitative guidelines for dosage individualization and optimization. Consequently it is of utmost importance in therapeutic drug monitoring. The aim of this paper is to present the background, the underlying conceptual theory, and the utility of the nonlinear mixed effects modeling approach. A special attention is put on the development of covariate sub-models, which aim to identify and quantify the sources of interindividual variability in pharmacokinetics. Finally, examples of how nonlinear mixed effects modeling can be applied to therapeutic drug monitoring in routine care of patients with epilepsy are presented.

Keywords: Nonlinear mixed effects model, Population pharmacokinetics, Therapeutic drug monitoring, Individual drug dosing

Presenting Author's biography

Iztok Grabnar was born in 1971 in Ljubljana, Slovenia. In 2000 he obtained a PhD degree at the Faculty of Pharmacy of the University of Ljubljana. Since 2006 he holds a position of an assistant professor at the Faculty of pharmacy of the University of Ljubljana. His work is focused on modeling and simulation of biomedical systems and biostatistics and their application to studies in the fields of pharmaceutical technology, biopharmaceutics, pharmacokinetics, and clinical pharmacology.

1 Introduction

Pharmacokinetics is the science of the kinetics of drug absorption, distribution and elimination, i.e. excretion and metabolism. It is one of the many biomedical disciplines that contribute to the discovery, development, and use of drugs. Characterization of pharmacokinetics is an important prerequisite for determination or modification of drug dosing regimens for individuals and groups of patients. The study of pharmacokinetics involves both, experimental and theoretical approaches. The experimental aspect involves the development of biologic sampling techniques, analytical methods for the measurement of drugs and metabolites in biological samples, and procedures that facilitate data collection and manipulation. The theoretical aspect of pharmacokinetics involves the development of mathematical models that predict drug disposition after drug administration. The application of statistics is an integral part of pharmacokinetic studies. Statistical methods are used for pharmacokinetic parameter estimation and data interpretation for the purpose of predicting and designing optimal dosage regimens. Statistical methods are applied to pharmacokinetic models to determine data error and structural deviations of the pharmacokinetic models [1].

During the drug development process, a large group of patients is tested to determine the optimum dosing regimens, which are then recommended by the manufacturer to produce the desired pharmacologic response in the majority of the intended patient population. However, inter- and intra-individual variability often result in lack of effectiveness due to sub-therapeutic drug concentration levels, or toxicity with drug concentration levels above the minimum toxic concentration. Both require adjustment to the dosing regimen. Clinical pharmacokinetics is the application of pharmacokinetic methods to drug therapy. It is a multidisciplinary approach to individually optimized dosing strategies based on the patient's disease state and all relevant patient-specific characteristics.

2 Population pharmacokinetics

Pharmacokinetic studies in patients have led to the appreciation of the large degree of variability in pharmacokinetic parameter estimates that exist across patients. Many studies have quantified the effects of factors such as age, sex, disease state, genetic phenotype, and concomitant drug therapy on the pharmacokinetics of drugs to account for the interindividual variability. Interindividual variability in pharmacokinetics is by many often viewed incorrectly as a nuisance factor that must be overcome, often through complex study designs, control schemes, and restrictive inclusion criteria. The

subjects of pharmacokinetic studies are usually a group of healthy volunteers or highly selected patients. Traditionally, the average behavior of a group has been the main focus of interest. Study design and selection of volunteers who are rigidly standardized so that they are as homogenous as possible are typical features of traditional pharmacokinetic studies. These studies are therefore often performed under artificial conditions not representative of the real situation in which the drug will be used. In contrast to traditional pharmacokinetic evaluation, population pharmacokinetic studies (i) aim to obtain relevant pharmacokinetic information in patients who are representative of the target population which is to be treated, (ii) recognize sources of variability such as inter- and intraindividual, and interoccasion, as important drug characteristics that should be identified, (iii) seek to explain variability by identifying demographic, biochemical, pathophysiologic, and genetic factors (covariates) that may influence the pharmacokinetic behavior, and (iv) they seek to quantitatively estimate the magnitude of the unexplained part of the variability in the patient population. The magnitude of the unexplained, i.e. random variability is important because the efficacy and safety of a drug may decrease as unexplained variability increases. Drug concentrations outside the target range become more likely, the greater the unexplained variability in the relationship between the dosage regimen and drug concentration. Sources of variability are usually categorized as interindividual and residual in nature. Residual variation includes intraindividual variability, i.e, random changes in a patient's pharmacokinetic parameters values over time, interoccasion variability, i.e, changes in a patient's parameters values from one occasion to another, drug concentration measurement error, dosing errors, errors in time recording, and model misspecifications.

2.1 Nonlinear mixed effects modeling

Nonlinear mixed effects models are similar to linear mixed effects models with the difference being that the function under consideration $f(x, \theta)$ is nonlinear in the model parameters θ. The model consists of two components, the structural model and the statistical or variance model. The structural model describes the mean response for the population. It is often found that the relationship between drug concentrations and time may be described by the sum of exponential terms. This lends itself to compartmental pharmacokinetic analysis in which the pharmacokinetics of a drug are characterized by representing the body as a system of well-stirred compartments with the rates of transfer between compartments following first-order kinetics. In the case of a drug that seems to be distributed homogenously in the body, a one-compartment model is appropriate and the relationship can be described by

the following monoexponential equation as shown in Eq. (1).

$$C_p = \frac{D}{V} e^{-\frac{CL}{V}t} \tag{1}$$

This equation describes the typical time course of the drug concentration in plasma (C_p) as a function of dose (D), time (t), drug elimination clearance (CL) and distribution volume (V). If one has an estimate of clearance and distribution volume, the plasma drug concentration can be predicted at different times after administration of any dose. The quantities that are known, because they are either measured or controlled, such as dose and time, are called fixed effects, in contrast to effects that are not known and are regarded as random. The parameters CL and V are called fixed effect parameters because they quantify the influence of the fixed effects on the dependant variable C_p [2].

Similar to a linear mixed effects models, nonlinear mixed effects models can be developed using a hierarchical approach. Data consist of an independent sample of n subjects with the i-th subject having n_i observations measured at time points $t_{i, 1}$, $t_{i, 2}$, …, $t_{i, ni}$. Let Y be the vector of observations, Y={ $Y_{1, 1}$, $Y_{1, 2}$, …, $Y_{n, 1}$, $Y_{n, 2}$, …, $Y_{n, ni}$}^{T} and let ε be the same size vector of random intraindividual errors. In the first stage (intraindividual variation level), the model describing how the mean profile changes over time is Eq. (2)

$$Y = f(x,t,\beta) + \varepsilon \tag{2}$$

where x is a matrix of fixed effect covariates specific to the subject, and β is a vector of estimable regression parameters. For simplicity, x will include t from this point. The regression function f depends on β in a nonlinear manner. At the second stage (interindividual variation level), the possibility that some of the β (denoted β_i, $\beta_i \in \beta$) can vary across individuals (with mean $\mu_{\beta i}$ and variance $\omega_{\beta i}^2$) is allowed, i.e. $\beta_i \sim (\mu_{\beta i}, \omega_{\beta i}^2)$, and can be explained by a set of subject specific covariates z. In other words, β_i is not fixed across subjects, but allowed to vary, and that variability may be explained by subject specific covariates. This stage is referred to as a covariate sub-model and relates how specific covariates (z) predict the subject-specific regression parameters β, Eq. (3)

$$\beta_i \approx \left(h(z,\theta), \omega_{\beta i}^2 \right) \tag{3}$$

where θ is a vector of estimable regression parameters. Collectively, the set of all $\omega_{\beta i}^2$ is referred to as the variance covariance matrix, denoted Ω. Those β that do not vary across individuals are refereed to as fixed effects, whereas those that do vary across individuals are referred as random effects. The structural model across all individuals is then Eq. (4)

$$Y = f(\beta,\theta,x,z) \tag{4}$$

For simplicity, the set of estimable regression parameters {β,θ} shall be denoted as θ.

As for the one-compartment example provided by Eq. (1), Y would be plasma drug concentration (C_p), x would consist of dose (D) and sample time (t), and β would consist of clearance (CL) and distribution volume (V), Eq. (5).

$$\beta = \begin{bmatrix} \mu_{CL} \\ \mu_V \end{bmatrix} \tag{5}$$

If clearance and volume of distribution were treated as correlated random effects, then ω_{CL}^2 and ω_V^2 denote the interindividual variability for clearance and volume of distribution, respectively, Eq. (6)

$$\Omega = \begin{bmatrix} \omega_{CL}^2 & \omega_{CL,V} \\ & \omega_V^2 \end{bmatrix} \tag{6}$$

where $\omega_{CL,V}$ is the covariance between clearance and distribution volume. Now further suppose that an individual's age was linearly related to clearance. Then clearance could be modeled as, Eq. (7)

$$CL_i = \theta_1 + \theta_2 \, Age_i + \eta_i \tag{7}$$

where CL_i is the average clearance for an individual with Age_i, θ_1 and θ_2 are the intercept and slope, respectively, Age_i is the age of the i-th subject, and η_i is the deviation of the i-th subject from the mean clearance of the population having Age_i. The ηs are assumed to be independent, have mean zero, and constant variance ω^2. In Eq. (4), age would comprise the set z. The hierarchical nonlinear mixed effects model can then be written as, Eq. (8)

$$\begin{bmatrix} CL \\ V \end{bmatrix} \approx \left(\begin{matrix} \theta_1 + \theta_2 \, Age & \begin{bmatrix} \omega_{CL}^2 & \omega_{CL,V} \\ & \omega_V^2 \end{bmatrix} \\ \mu_V & \end{matrix} \right)$$
$$Y = f(D,t,CL,V,Age) \tag{8}$$
$$= \frac{D}{V} e^{-\frac{\theta_1+\theta_2 \, Age}{V}t}$$

Residual variance models describe the random, unexplained variability in the regression function f. Hence, the structural model is extended to, Eq. (9)

$$Y = f(\theta,\phi,x,z,\varepsilon) \tag{9}$$

where ε is the residual term and ϕ are residual variance model parameters. Commonly used residual variance functions are additive, constant coefficient of variation or proportional, exponential, and combined additive and proportional model. Under all these models, the generic residuals are assumed to be independent, have zero mean, and constant variance σ^2.

Returning to the previous structural model, Eq. (1), a complete nonlinear mixed effects model can be written as, Eq. (10)

$$Y_{ij} = \frac{D_i}{V_i} e^{-\frac{(\theta_{1i}+\theta_{2i}\,Age_i+\eta_i)t_{ij}}{V_i}} + \varepsilon_{ij} \qquad (10)$$

where D_i is the dose administered to subject i, V_i is the distribution volume in i-th subject, clearance is modeled as a random effect that is a linear function of patient age, η_i the deviation of for the i-th subject from the population mean clearance such that η has mean zero and variance ω^2, t_{ij} is time, and ε is the residual deviation of the measured concentration with mean zero and variance σ^2.

2.2 Development of a population pharmacokinetic model

The NONMEM package continues to be the most widely used software for the population pharmacokinetic analysis. Its limitations lie mostly with its user interface, which, despite the numerous modifications to the code including the NM-TRAN preprocessor, remains a warehouse of FORTRAN 77 subroutines. Recently many application programming interfaces to NONMEM have been developed, such as Wings for NONMEM, PDx-Pop, and Perl-speaks-NONMEM. Additionally, several alternatives to NONMEM are available and some are still under development. The available software for population pharmacokinetic analysis has been reviewed by Aarons [3]. All software alternatives and methods are based on a hierarchical nonlinear mixed effects modeling approach described in a previous section. NONMEM is especially useful for sparse, randomly collected data. Although the data are pooled into one data set the individuals are still identifiable, which permits different numbers of repeated measurements per subject. The inclusion of covariates during the estimation procedure offsets unbalanced data. NONMEM is able to derive population models when only a few samples are available from each individual. Consequently, this approach is ideal for studying populations, such as the very old, very young or very sick, which are most difficult to address. Although the study design does not call for the collection of samples at specific times, it is important to note that some thought must be given to optimal collection times. A certain pharmacokinetic parameter can not be calculated with any degree of precision unless data are available that reflect that parameter.

The first step in the development of a population pharmacokinetic model is to identify the base or structural model, which is the model that best describes the data in the absence of covariates. It may be, however, that a covariate has such a profound influence on a particular model parameter, one may choose to include that covariate into the base model from the start. For instance if a drug is eliminated from the body exclusively by the kidney, such as the aminoglycoside antibiotic gentamicine, then creatinine clearance (CL_{Cr}) may be very highly correlated with the drug clearance and CL_{Cr} may be built into the model from the beginning stages. If previous studies have identified a structural model one typically proceeds from there. In the absence of a known base model, a variety of candidate base models, one-, two-, and three-compartment models with different absorption models, if the drug is given by extravascular administration are tried, and the best model is chosen using a combination of likelihood ratio test, Akaike information criterion, and graphical examination using goodness of fit plots.

2.2.1 Covariate model building

Once the base model is identified, covariate sub-models are developed. A covariate is any variable that is specific to an individual and may influence the pharmacokinetics of a drug. Covariates are classified as intrinsic factors (inherited, genetically determined), such as age weight height, and race, or extrinsic factors (subject to outside environmental influences), such as dose, degree of drug compliance, smoking status, and presence of concomitant medications. In general intrinsic factors do not change over short spans of time or at all, whereas extrinsic covariates may change many times during a study. Covariates may also be classified as either continuous (such as age), dichotomous (such as sex), or polychotomous/categorical (such as race). One of the biggest reasons for using a population approach to modeling pharmacokinetics is that subject-specific characteristics can be built into the model through their associations with model parameters. For example, if distribution volume is dependent on weight then weight can be built into the model, therby reducing both, interindividual variability in volume of distribution and unexplained, residual variability. When covariates are identified and the interindividual variability is sufficiently reduced, individualized dosage regimens become possible [2].

In general, how a covariate is built into the model is dependent on the type of variable the covariate is. For continuous covariates, covariate sub-models are generally the three different functions, linear, exponential, or power. Choosing the function broadly depends on the modeling approach taken. One such approach is covariate screening, in which given the population pharmacokinetic model the Empirical Bayesian Estimate (EBE) of the pharmacokinetic parameter is estimated for each subject. The EBEs are then plotted against subject specific covariates and examined for a relationship between the two. If examination of the scatter plot shows a straight line, a linear model is used. However, if the plot shows curvature then exponential or power models are often used. More formally a regression line or generalized additive model (GAM) can be used to statistically test

for curvature, nonlinearity or break points. A judgment is then made by the modeler as to the nature of the relationship between covariate and EBE and this relationship is taken forward into the covariate sub-model. When the covariate is categorical and dichotomous three possible models are typically used: additive, fractional change, and exponential.

The number of parameter - covariate combinations is often large (for example, 4 model parameters and 20 covariates equal 80 potential combinations) and the task of identifying the relevant ones can be a time consuming exercise. An efficient approach to accomplish this is to use Stepwise Covariate Model building (SCM) directly in NONMEM [2]. This procedure is also known as (orthogonal) Forward Selection—Backward Elimination. Stepwise procedures in general have been shown to exhibit a risk of including false parameter - covariate relations, of giving rise to biased estimates of the included relations as well as of yielding too narrow confidence limits [2]. Other studies have reported that these problems may not be large for pharmacokinetic models [2]. SCM assumes that there exists a *structural* model, i.e. a model that relates the main response variable, for example drug concentrations in pharmacokinetics, to the main independent variable, for example time since drug administration. The task of SCM is to identify the covariates that explain variability in the parameters of the structural model. In a first step, each relevant parameter - covariate combination is added and estimated one by one in the structural model. The model with the largest improvement over the starting model is retained as the starting model for the next step. In each subsequent step the remaining parameter - covariate combinations are tried. This forward inclusion is continued until no improvement can be gained by adding new model components. The measure of model improvement is usually based on statistical significance. Optionally, the forward inclusion step can be followed by a backward elimination step. This proceeds according to the same general scheme as the forward step, but reversely, using stricter improvement criteria. This adaptive procedure for covariate model building relies heavily on the validity of the statistics used for model discrimination.

2.2.2 Estimation methods

NONMEM version 5 and higher offers two general approaches towards parameter estimation with nonlinear mixed effects models, first-order approximation (FO) and first-order conditional estimation (FOCE), with FOCE being more accurate and computationally difficult than FO. FO was the first algorithm derived to estimate parameters in a nonlinear mixed effects model and was originally developed by Sheiner and Beal [4]. FO approximation expands the nonlinear mixed effects model as a first-order Taylor series approximation about $\eta=0$ and then estimates the model parameters on the linear approximation to the nonlinear model. FOCE on the other hand is a first order approximation of the nonlinear mixed effects model around the posterior mode of η. Since FOCE depends on a conditional estimate of η, the method is referred as conditional estimation algorithm.

2.2.3 Precision of parameter estimates and confidence intervals

The aim of a population pharmacokinetic analysis is to estimate the population parameters and associated variance components. Additionally the precision of these estimates, i.e. standard errors are sought as small standard errors are indicative of good parameter estimation. Estimation of the standard errors of the model parameters is usually based on standard maximum likelihood theory assuming the number of individuals used in the estimation is large and the random effects and residual errors are normally distributed, i.e. the standard errors are asymptotically normally distributed. In NONMEM, the default covariance matrix is a function of the Hessian and the cross-product gradient of the -2 log-likelihood (-2LL) function. The standard errors are computed as the square root of the diagonals of this matrix. An approximate asymptotic confidence interval can then be generated using a standard normal distribution (large sample) approximation. A number of problems are noted with this approach. Consequently, in most cases the resulting estimates of the standard errors can only be used qualitatively.

Alternative methods to estimate standard errors are the nonparametric bootstrap method and the likelihood profiling. The Bootstrap [5] is a general method for measuring statistical accuracy and precision. Briefly, it involves creating ''new'' data sets by sampling with replacement from the original data and applying the same analysis steps to each of the new data sets as was performed on the original data. The results from the new data sets form distributions, which reflect the uncertainty in the original analysis. These distributions can be used to assess covariate selection stability [6], uncertainty of parameter estimates [7] and to correct for certain types of bias [8]. The resampling is performed with replacement on statistically independent parts of the data, which in population pharmacokinetics usually corresponds to individuals. Depending on what the statistic of interest is, different numbers of resampled data sets are needed. Bias correction of parameter estimates typically needs 50 bootstrap data sets, whereas estimation of standard errors requires 200. To calculate 95% confidence intervals approximately 2000 bootstrap data sets is required.

Log-likelihood profiling is one alternative method where no assumption regarding symmetry of the interval has to be made [9,10]. Fixing a parameter to values close to the estimate obtained from a maximum

likelihood procedure (as the one implemented in NONMEM) and refitting this reduced model generates a likelihood profile. Often, as is the case in NONMEM, minus two times the natural logarithm of the likelihood is used and the maximum likelihood then corresponds to the minimum of this quantity. If a parameter is fixed, this model can be regarded as an alternative, competing with the full non-fixed model in being the most appropriate for describing the data at hand. The rival models are nested and the difference in the log-likelihoods of the data for the two models is approximately χ^2-distributed. At p=0.05, a statistically significantly improvement is achieved when the log-likelihood difference is 3.84.

3   Advantages of the population pharmacokinetic modeling approach

There are many advantages of population pharmacokinetics compared with the traditional approach to studying pharmacokinetics. Unlike the traditional studies in which subjects are sampled intensively, the population approach to evaluating the pharmacokinetics of a drug allows both sparsely and intensively sampled data to be used. Population pharmacokinetics enables the execution of pharmacokinetic investigations in special populations such as neonates and critical care patients where the number of samples to be obtained per subject is limited because of ethical and medical concerns. During drug development, relatively few samples can be obtained from patients participating in Phase II and III studies for the determination of the pharmacokinetics of a drug in the relevant population and for the determination of the relationship between dose, exposure, and efficacy/safety. Additionally, this approach yields better estimates of interindividual variability than traditional approaches that yield positively biased estimates [2]. A combination of accurate and precise estimates of interindividual variability and the mean parameter value for a drug is useful for selecting an initial dose strategy for drug therapy in a patient, and allows Bayesian feedback analysis to be performed for dosage individualization. Additionally, the analyses of sparse samples collected for population pharmacokinetic analysis have been reported to be cost-effective [2]. The population pharmacokinetic approach also allows one to combine heterogenous types of data from varying sources. For example, one could pool data from several different trials, study centers, variable biomatrices (plasma and serum), intense plus sparsely sampled populations, or experimental plus observational data. The combining of differing data sets often increases power to identify multi-compartment or nonlinear models, incorporate additional covariates, or gain precision in the estimation of the model.

4   Clinical application to therapeutic drug monitoring in epilepsy

Therapeutic drug monitoring (TDM) is the measurement and clinical use of drug concentrations in the body fluids, i.e. plasma or serum, to adjust drug dosage and schedule to each patient's individual therapeutic requirements. It has been successfully implemented in the therapy of various diseases, among them epilepsy was one of the first to benefit of it. The management of epilepsy on clinical grounds only, can be problematic. The main reasons for this are that: i) antiepileptic drug (AED) treatment is prophylactic and seizures occur at irregular intervals, consequently it is difficult to determine whether the administered dose is sufficient for long-term seizure control; ii) clinical symptoms and signs of toxicity may be subtle, or difficult to differentiate from the manifestation of underlying disorder; and iii) there are no direct laboratory markers for clinical efficacy or for the manifestation of most common AED toxicity or adverse CNS effects.

The basic ground for implementing TDM in epilepsy is the assumption that drug concentration rather than drug dosage are better correlated with clinical effects. Although, the indications for TDM usefulness in the management of epilepsy are similar for all AEDs, the TDM is likely to be of particular value for the drugs that exhibit pronounced variability in pharmacokinetics due to drug-drug interactions, and for AEDs with established relationship between drug concentration and therapeutic or toxic effects, narrow reference range, reversible action without development of tolerance, and with activity per se not through metabolites, unless these are measured.

Carbamazepine (CBZ) is one of the oldest AEDs and still the drug of choice for treatment of simple or complex partial and generalized tonic-clonic seizures. CBZ absorption is relatively slow, variable and formulation dependent. Bioavalability is assumed to be 75 - 85% and apparent volume of distribution (V/F) after peroral administration varies from 0.2 to 2 L/kg. CBZ undergoes extensive metabolism, with major pathway involving cytochrome P450 (CYP) 3A4, to equipotent carbamazepine-10-11 epoxide. CBZ induces many enzyme systems including CYP 1A2, 2C and 3A and glucuronosyltransferase. Consequently, it increases the metabolism of many drugs, including its own. Additionally, a number of AEDs or non-AEDs may induce or inhibit CBZ metabolism during polytherapy.

Valproic acid (VPA) has wide anticonvulsive effects used in treatment of partial and generalized forms of epilepsy. VPA is almost completely absorbed from gastrointestinal tract, highly bound to plasma albumin and shows dose-dependent kinetics at high doses. Apparent volume of distribution (V/F) is 0.1-0.5 L/kg. VPA is primarily eliminated via hepatic metabolism mainly by conjugation with glucuronic acid, β-

oxidation and ω-oxidation. It undergoes metabolism via glucuronidation or CYP 2C9, 2C19, and 2A6 izoenzymes, therefore its metabolism can be altered by other drugs. Drugs which interact with VPA include both, AEDs such as CBZ, phenobarbital, topiramate, and lamotrigine, as well as non-AEDs.

Topiramate (TPR) belongs to the second generation of AEDs and has been approved for treatment of adults and children with different kinds of epilepsy either as mono or as adjunctive therapy. Following oral administration of TPR, absorption is rapid and almost complete with bioavailability ranging from 81 to 95%. In the dose range from 100 to 1200 mg/day the mean V/F is between 0.6 and 1.0 L/kg. Over 80% of TPR is eliminated via the kidneys, predominantly as unchanged drug. To date, six trace metabolites formed by glucuronidation, hydroxylation and hydrolysis have been identified in humans. The most important drug-drug interactions occur with hepatic-enzyme-inducing AEDs, i.e. CBZ and VPA, which decrease TPR plasma concentrations by 40 and 17%, respectively.

Clinical experience has demonstrated that individualized dose adjustment by the aid of TDM can significantly improve treatment with AEDs. Development of a population pharmacokinetic model is a logical extension of TDM, because it allows estimation of individual patients' pharmacokinetic parameters based on sparse concentration measurements and ultimately effective control of dosing. In addition to being a valuable tool in designing a safe and effective dosing regimen for patients with epilepsy, population pharmacokinetic models permit evaluation of various factors that can influence PK characteristics.

The subsequent sections of this paper present the summaries of our population pharmacokinetic studies with carbamazepine [11], valproic acid [12] and topiramate [13] aiming to evaluate the influences of various demographic and biochemical factors, and concomitant drug treatment on pharmacokinetics.

4.1 Modeling methods

Pharmacokinetic analysis of the population data was performed using NONMEM software package. The structural model used to fit the concentration-time data was a one-compartment model with first-order absorption and elimination as implemented in ADVAN2/TRANS2 PREDPP subroutine. In most cases only one or in some cases two drug blood concentrations per patient were determined. With such sparse data there are many problems with parameter estimation. The estimates are commonly biased and their precision is low, especially when all parameters are estimated. However, the average steady-state drug concentration depends on apparent oral clearance (CL/F), which is usually adequately predicted. In the present studies, estimation of V/F and absorption rate constant (k_a) was not possible. Therefore, V/F and k_a

had to be fixed to a literature value. k_a was estimated using the following relationship: $t_{max}=\ln(k_a/k_e)/(k_a-k_e)$, based on a literature value of elimination rate constant (k_e), and t_{max}. In the first stage of model building the base model was derived. To describe interindividual variability of CL/F ($\omega^2_{CL/F}$) exponential model was used, while an additive, proportional, and combination error models were tested for residual variability of drug concentration (σ^2). The model appropriateness was evaluated by standard diagnostic plots. Additional criteria were convergence of minimization, number of significant digits more than 3, successful covariance step and gradients in the final iteration in the range between 10^{-3} and 10^2. Alternative models were compared by the likelihood ratio test. Criterion for selection of a model was a change in minimum value of objective function -2LL (ΔOFV), of at least 3.84 per one additional parameter, corresponding to p < 0.05. In the following step, covariate model was developed using a forward inclusion method. Continuous covariates including patients' weight (WT), body surface area (BSA), age, and daily dose of the investigated drug and co-treated drugs were included into the base model using a linear and power relationship in a mean centered manner. Among the categorical covariates tested were patients' sex, effect of tobacco smoking (TOB) and concomitant medications. Effect of each covariate was tested against the base model. Significant covariates according to the likelihood ratio test were rank-ordered and one by one introduced into the full model. The final model was determined by testing each covariate against the full model using a likelihood ratio test to see if it should remain in the model. Additional criterion for the retention of a covariate in the model was reduction in the unexplained interindividual variability and improvement in the precision of the parameter estimates. In each step of the model building process, improvement of the model was assessed by the goodness-of-fit plots, including the agreement between the observed and predicted plasma concentrations, reduction in the range of conditional weighted residuals, and uniformity of the distribution of the conditional weighted residuals vs. the predicted concentrations. A bootstrap sampling method with replacement was applied to calculate 95% confidence intervals of the final model parameter values.

4.2 Carbamazepine

The CBZ study data from 311 patients were systematically explored and the influence of various covariates on CBZ pharmacokinetics using population approach was evaluated [11]. Univariate analysis of covariate relationships performed by forward inclusion into the base model revealed that TOB, sex, co-therapy with lamotrigine (LTG) and benzodiazepines (BDZ) have no effect on CL/F of CBZ. On the other hand, inclusion of WT, age, CBZ dose (DCBZ), phenobarbitone dose (DPB), VPA dose

(DVPA) and CBZ tablet formulation significantly improved OFV and reduced unexplained interindividual variability. Furthermore, the relationship between CL/F and WT, AGE and DCBZ was best described by the power model, while the relationship with DPB was linear rather than power. In addition, incorporating DVPA in both a linear and a power manner did not significantly improve the data fitting, but as indicated by the plots of post-hoc Bayesian estimates, CL/F was significantly higher, if DVPA was greater than 750 mg/day. In the backward elimination step the influence of age on CL/F was removed. Parameters of the final model are presented in Table 1.

Tab. 3 Estimates of the final population pharmacokinetic model of carbamazepine. DCBZ carbamazepine dose; DPB phenobarbitone dose; WT weight; VPA valproic acid

Parameter	Estimated value	95% Confidence interval
θ_{CL}	5.35	4.95 - 5.75
$\theta_{CL,DCBZ}$	0.591	0.499 - 0.683
$\theta_{CL,DPB}$	0.414	0.238 - 0.590
$\theta_{CL,WT}$	0.564	0.362 - 0.766
$\theta_{CL,VPA}$	1.18	1.03 - 1.33
$\omega_{CL/F}$ [CV%]	36.5	31.6 - 40.7
σ [µg/mL]	1.18	0.98 - 1.36

The final model is described by the following equation, Eq. (11)

$$CL/F\,[L/h] = 5.35 \cdot \left(\frac{DCBZ\,[mg/day/kg]}{15} \right)^{0.591} \cdot$$
$$\left(1 + 0.414 \cdot \frac{DPB\,[mg/day/kg]}{2} \right) \cdot \qquad (11)$$
$$\left(\frac{WT\,[kg]}{70} \right)^{0.564} \cdot 1.18^{VPA}$$

where VPA is 1 if DVPA>750 mg or 0 otherwise. Final model revealed positive correlation between DCBZ and CL/F (Figure 1). The relationship was nonlinear and of utmost importance in adjusting CBZ dose in the post-induction phase. Possible explanations for the effect of dose on CL/F have included decreased CBZ bioavailability and increased clearance due to autoinduction of metabolism at higher CBZ doses, and TDM effect. To our knowledge this is the only study in which the influence of DPB on CBZ CL/F was investigated, in contrast to previous population pharmacokinetic studies where co-therapy with PB was considered only as a categorical covariate. Due to PB induced CBZ metabolism an increase in CBZ CL/F in the range from 16 to 44% is reported. According to the results

of our study the relationship between CBZ CL/F and DPB is linear. Based on the results of the final population model an increase in CBZ CL/F by 30% in concomitant therapy with PB 100 mg daily and 44% with 150 mg daily is estimated for a patient with an average WT of 70 kg. However, in view of the fact that the main CBZ metabolite CBZE contributes to both the therapeutic and toxic effects of the drug, its concentration should have been monitored in order to evaluate clinical importance of this drug-drug interaction. As reported in other studies VPA increases CL/F of CBZ by 7 to 23%. In our study CL/F of CBZ was 18% higher in patients co-treated with VPA, if DVPA was greater than 750 mg/day. Since total (bound + free) drug concentration was measured in our study and due to the fact that VPA is highly bound to plasma proteins, this slight increase in CBZ CL/F may be caused by displacement from plasma proteins.
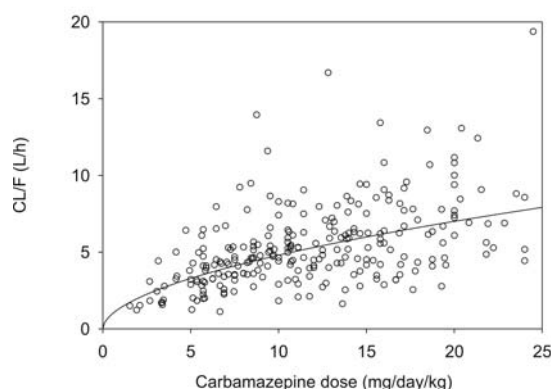

Fig. 1 Relationship between individual estimates of carbamazepine peroral clearance and daily dose

4.3 Valproic acid

The study evaluated VPA CL/F from 153 patients with diagnosed epilepsy receiving VPA either as mono antiepileptic therapy or in combination with other AEDs [12]. Interindividual variability of CL/F was evaluated by an exponential model, while residual variability in VPA concentrations was most adequately described by a combination error model comprising proportional and additive component. Analysis of the plots of Bayesian estimates of individual patient's CL/F versus various covariates indicated a step-like increase of VPA CL/F with DVPA greater than 1000 mg/day, while CL/F continuously increased with patients' WT. Moreover, CL/F in patients co-treated with TPR was lower compared to the group of patients on monotherapy with VPA and combination therapy with other AEDs. The univariate analysis of covariate relationships revealed that inclusion of DVPA greater than 1000 mg/day into the base model, resulted in the highest decrease in OFV of 16.809 (p < 0.0001). Additionally, the influences of patients' WT (ΔOFV=9.945, p =

0.002) and co-treatment with TPR (ΔOFV=4.784, p = 0.029) were found significant and were introduced into the full model. In the backward elimination step no covariate was removed from the full model. The final population model parameter estimates are presented in Table 2.

Tab. 2 Parameters estimates of the final population pharmacokinetic model of valproic acid in patients with epilepsy

Parameter	Estimated value	95% Confidence interval
θ_{CL}	0.517	0.470 - 0.568
$\theta_{CL,WT}$	0.556	0.176 - 0.885
$\theta_{CL,VPA}$	1.43	1.24 - 1.63
$\theta_{CL,TPR}$	0.765	0.617 - 0.994
$\omega_{CL/F}$ [CV%]	31.9	22.4 - 37.9
σ_a [mg/L]	13.2	3.17 - 18.8
σ_p [%]	23.8	15.4 - 32.4

The model is described by the following equation, Eq. (12)

$$CL/F[L/h] = 0.517 \cdot \left(\frac{WT[kg]}{70} \right)^{0.556} \cdot \qquad (12)$$
$$1.43^{VPA} \cdot 0.765^{TPR}$$

where VPA is 1 if dose is greater than 1000 mg/day, or 0 otherwise, and TPR equals 1 in patients co-treated with TPR, or 0 if not. VPA CL/F positively correlated with patient's WT, with the exponent (95% Cl) of 0.556 (0.176 - 0.885). The increase of CL/F with DVPA greater than 1000 mg/day is in accordance with the results of other studies investigating the effect of DVPA on CL/F. Adjunctive therapy with TPR or LTG is common in patients who do not respond to mono therapy with VPA, and there is uncertainty about the effect of TPR or LTG on VPA CL/F. Co-therapy with TPR was included in the final population model, showing 23% (0.6-38.3%) decrease in VPA CL/F in patients co-treated with TPR. Results of the studies investigating VPA interaction with TPR are inconsistent as in some studies an increase in VPA CL/F was observed with TPR co-treatment. This can be explained with the multiple effects of TPR on metabolic pathways of VPA. Namely, formation clearance of VPA glucuronide was found to decrease by 35%, while β-oxidation was found to increase by 42%, during co-treatment with TPR. No influence of co-treatment with LTG, BZD, CBZ and PB on VPA CL/F was detected, although a trend of 14.9 and 6.9% decrease in VPA concentrations in patients concomitantly treated with CBZ and PB, respectively, was observed in comparison to patients on mono therapy.

4.4 Topiramate

As the number of patients included in this study [13] was relatively low – 26, k_a was fixed which enabled other parameters (CL/F, V/F) to be adequately estimated. Interindividual variability of CL/F was modeled with an exponential model, while residual intraindividual variability of topiramate concentration was most adequately described by the additive error model. Analysis of the plots of Bayesian estimates of individual patient's CL/F obtained with the base model versus various covariates indicated an increase of CL/F with patient age. Additionally, mean topiramate CL/F in patients not co-treated with enzyme inducing AEDs (1.61 L/h) was lower compared to patients co-treated with CBZ (2.08 L/h), while in one patient co-treated with phenobarbital CL/F was 1.06 L/h.

Inclusion of the influence of age on CL/F into the base model decreased OFV by 4.823 (p = 0.028) and reduced unexplained interindividual variability of CL/F to 44.3%. Additionally, the influence of co-treatment with CBZ (ΔOFV = -5.930, p = 0.015) on topiramate CL/F was found significant and was introduced into the full model. Since the mean age of patients co-treated with CBZ was lower, we presume that the effect of CBZ co-treatment on topiramate CL/F was confounded with the patients' age when tested against the base model.

Tab. 3 Estimates of the final population pharmacokinetic model of topiramate

Parameter	Estimated Value	[a]95% Confidence interval
$\theta_{CL/F}$	1.47	1.18-1.86
$\theta_{CL/F, Age}$	0.421	0.177-0.755
$\theta_{CL/F, CBZ}$	1.70	1.31-2.23
$\theta_{V/F}$	0.518	0.419-0.633
$\omega_{CL/F}$ [CV%]	39.2	22.5-49.1
σ [%]	13.8	8.7-18.0

[a]2.5 and 97.5 percentile of the parameter estimates over 1000 bootstrap samples

In the backward elimination step no covariate effect was removed from the full model. The final model is described by the following equations, Eq. (13)

$$CL/F[L/h] = 1.47 \cdot 1.70^{CBZ} \cdot \left(\frac{Age[yrs]}{30} \right)^{0.421} \qquad (13)$$
$$V/F[L] = 0.518 \cdot WT[kg]$$

where CBZ is 1 in patients co-treated with carbamazepine, or 0 otherwise. Parameters of the final model are presented in Table 3.

Under the conditions of our study topiramate CL/F was found to increase with patient's age and co-therapy with carbamazepine (Figure 2).
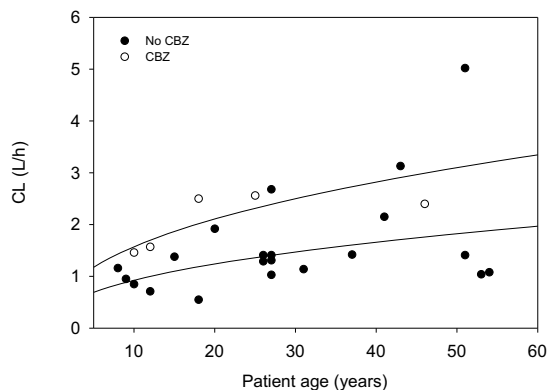
Fig. 2 Relationship between individual estimates of topiramate clearance, patient's age and co-treatment with carbamazepine (CBZ)

Topiramate oral clearance was 70% higher in patients co-treated with carbamazepine compared to patients on topiramate monotherapy. Previous studies demonstrate that co-treatment with enzyme-inducing AEDs (carbamazepine, phenytoin, phenobarbital, primidone) enhances hepatic metabolism of topiramate. Enzyme induction by CBZ is associated with an approximately 2 to 3-fold increase in topiramate oral clearance, mostly due to an elevation in metabolic clearance. In contrast to CBZ, BZD, VPA and risperidone co-therapy did not exert a significant influence on topiramate pharmacokinetics. Although relationship between plasma topiramate concentrations and occurrence of adverse events related to CNS was demonstrated previously, in the present study only incidence of headache was associated with the average steady-state plasma concentration of topiramate. Lower plasma concentration of topiramate was observed in patients experiencing headaches compared to patients absent from this adverse event. This could be attributed to the antimigraine action of topiramate. On the other hand, somnolence, ataxia, tremor, speech disorders and fatigue were associated with adjunctive therapy with carbamazepine, valproic acid, benzodiazepines, and risperidone.

4.4 Conclusion

The population pharmacokinetic models developed in these studies can be used for Bayesian estimation of pharmacokinetic parameters in individual patients based on sparse concentration measurements and for selection of optimum dosing regimen in routine patient care.

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