Prediction of Pharmacokinetic Parameters and the Assessment of Their Variability in Bioequivalence Studies by Artificial Neural Networks

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Purpose. The methodology of predicting the pharmacokinetic parameters (AUC, c_{max}, t_{max}) and the assessment of their variability in bioequivalence studies has been developed with the use of artificial neural networks.

Methods. The data sets included results of 3 distinct bioequivalence studies of oral verapamil products, involving a total of 98 subjects and 312 drug applications. The modeling process involved building feedforward/backpropagation neural networks. Models for pharmacokinetic parameter prediction were also used for the assessment of their variability and for detecting the most influential variables for selected pharmacokinetic parameters. Variables of input neurons based on logistic parameters of the bioequivalence study, clinical-biochemical parameters, and the physical examination of individuals.

Results. The average absolute prediction errors of the neural networks for AUC, c_{max}, and t_{max} prediction were: 30.54%, 39.56% and 30.74%, respectively. A sensitivity analysis demonstrated that for verapamil the three most influential variables assigned to input neurons were: total protein concentration, aspartate aminotransferase (AST) levels, and heart-rate for AUC, AST levels, total proteins and alanine aminotransferase (ALT) levels, for c_{max}, and the presence of food, blood pressure, and body-frame for t_{max}.

Conclusions. The developed methodology could supply inclusion or exclusion criteria for subjects to be included in bioequivalence studies.

KEY WORDS: bioequivalence; neural networks; prediction; pharmacokinetics; verapamil.

INTRODUCTION

Bioequivalence studies are finance and time-consuming for the investigator, which is why their eventual negative results are undesirable for both investigator and volunteer or patient. The variability of pharmacokinetic parameters (AUC, c_{max}, t_{max}), which are usually calculated to determine the rate and extent of drug absorption, has a great influence on the study results (1). The values of pharmacokinetic parameters were predicted by using artificial neural networks (2–6), and the variability of pharmacokinetic parameters were calculated from their values. We attempted to find a new way to reduce the variability of pharmacokinetic parameters with a proper selection of individuals. We were seeking variability sources between clinical-biochemical parameters and the physical examination of individuals, formulation properties, and the study design. The most influential variables were determined by performing a sensitivity analysis of the neural models. The known variability sources and predicted values of pharmacokinetic parameters could help to determine individuals with a higher intra- and interindividual variability contribution in order to review their inclusion in the study. Verapamil was used as a model drug where it was also interesting as a member of the highly variable drugs group.

MATERIALS AND METHODS

Database

The database included results of 3 distinct bioequivalence studies of two oral verapamil products. A 2 × 2 cross-over design was used in all the studies, but they differed in the number of individuals involved, the number of applications (single-multiple dose), and the presence of food relative to drug administration. In total, 98 subjects and 312 drug administrations were involved in the data sets. Both products were the same in all the studies, whose sponsor was the pharmaceutical company Lek d.d, Ljubljana.

In a multiple dose study we used AUC, which was calculated after the last dose application. The concentration at time zero (t_0) equaled 0 in single dose studies, whereas it was different from 0 in a multiple dose study. Some of the variables used for building models were nominal and we transformed them to numeric values. Our data were variable and neural networks predicted values that rarely occur in a training matrix with a higher prediction error. For these reasons, we divided the database into 10 similar operational data sets (7). They were similar in the pharmacokinetic parameter value and the observations of one individual were united in a particular data set. One of the data sets was used as a test set for the validation of network prediction and the other 9 as a training set. In this way, 10% of the whole database was used for testing the utility of the model (8). Each data set was once a test set and for this reason, 10 neural networks were constructed for each pharmacokinetic parameter. Before the division of the database, we excluded 5 randomly assigned individuals, and after the division these 5 individuals were added to each test set. These individuals were used for comparison of the prediction between all 10 neural networks for the same pharmacokinetic parameter. Furthermore, a randomly assigned test set of 14 individuals was excluded from the database before training and was called a random test set. It was also used for the validation of the neural networks prediction.

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ABBREVIATIONS: AUC, area under concentration-time curve; c_{max}, peak plasma concentration; t_{max}, time to reach peak plasma concentration; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ME, mean error; MSE, mean squared error; RMSE, root mean squared error; |Le%|, absolute learning error percent; |Pe%|, absolute prediction error percent; t_0, time of dose application after which pharmacokinetic parameters were determined; CV_m, coefficient of variation for measured values; CV_p, coefficient of variation for predicted values; AbsAveSens, absolute average sensitivity; i, i-th input; n, number of training rows; FF, evaluation of the network; I, current value of input in training row (in internal units); Δ, 0.1; CV, coefficient of variation; u, parameter of input influence.

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Building Neural Networks

Feedforward/backpropagation neural networks with a sigmoid transfer function and one hidden layer were created with NNMODEL (Version 1.402, Neural Fusion) neural network software. We used supervised learning with the delta learning rule and its moment version (9–12). The hidden and output neurons received an additional constant input that is called the bias (13). The input neurons were introduced with values of 19 variables: the name of the individual, height, age, body weight, heart rate, constitution, blood pressure, serum creatinine, serum bilirubine, serum calcium, serum concentration of alkaline-phosphatase, serum AST levels, serum ALT levels, serum total proteins, concentration in time zero, presence of food, sequence, period, and formulation. Networks for AUC, c_{max}, and t_{max} prediction included 9, 10, and 10 hidden neurons, respectively. The output neuron was one of the pharmacokinetic parameters (AUC, c_{max}, or t_{max}).

Training Neural Networks

During the training of neural networks, the training status graph was updated after each iteration. The status graph displayed the sum squared error of the training and test matrix. An overtraining phenomenon occurs after 200 iterations, and for this reason we stopped training at this point (16).

Validation of Neural Networks

For all neural networks predicted output values were calculated by NNCALC Excel-add in (Neural Fusion). From the predicted vs. the obtained differences of pharmacokinetic parameter values, mean error (ME), mean squared error (MSE), root mean squared error (RMSE), absolute learning error percent (|Le%|), and absolute prediction error percent |Pe%| were calculated for learning and prediction validation (3,14):

$$ME = \frac{1}{N}\sum_{i=1}^{N} Pe_i;\ Pe = \text{observed} - \text{predicted};$$

$$N = \text{number of observations} \quad (1)$$

$$MSE = \frac{1}{N}\sum_{i=1}^{N} Pe_i^2;\ RMSE = \sqrt{MSE} \quad (2)$$

$$|Pe\%| = 100\frac{|Pe|}{\text{prediction}} \quad (3)$$

The absolute learning error percent was calculated in the same way as absolute prediction error percent, only the training set was used for calculations instead of the test set. Measured and predicted graphs were also constructed for all observations (i.e., drug administrations in individuals), and for the validation of prediction, the coefficient of variation for both measured and predicted values of pharmacokinetic parameters was calculated. When logarithmic values of pharmacokinetic parameters were normally distributed (log AUC, log c_{max}), the t-test and F-test were performed at alpha level 0.05 for the comparison of measured and predicted average and variances of output values of the test sets. In the case of a non-normally distributed parameter t_{max}, non-parametric Kruskal-Wallis ANOVA was performed.

Sensitivity Analysis

A sensitivity analysis was performed with the NNMODEL. It showed the sensitivity of the output variables to changes in the input variables. The sensitivity is calculated by summing the changes in the output variables caused by moving the input variables by a small amount over the entire training set. We used absolute average sensitivity (AbsAveSens) for sensitivity analysis (15):

$$AbsAveSens_i = \sum_{j=1}^{n} |FF(I_{ij} + \Delta) - FF(I_{ij} - \Delta)| \quad (4)$$

The most influential variables for pharmacokinetic parameters were determined by performing a sensitivity analysis for all neural networks. To determine the average sensitivity of one pharmacokinetic parameter, we added $AbsAveSens_i$ of all 10 neural networks for each input variable. The total was then divided by 10. Input variable sensitivity and variability are important for the variability of pharmacokinetic parameters. Therefore we multiplied $AbsAveSens_i$ and the coefficient of variation of a particular input variable. A new multiplied parameter was named u:

$$u_i = AbsAveSens_i * CV_i \quad (5)$$

On the basis of the average sensitivity and the parameter u, input variables were sorted in order of influence on the pharmacokinetic parameter value.

Linear Regression Models

A correlation analysis was performed for all data sets. For input variables with a statistical significant Pearson correlation coefficient, linear regression models were constructed. The difference in coefficient of variation for measured and predicted values was calculated and compared with neural models.

RESULTS

All results are presented for normalized variables. Table I shows the validation of networks learning for each pharmacokinetic parameter. The average ME of all networks is negative, which means employed neural networks generally predicted

Table I. Validation of Networks Learning Performance

	ME[a]	MSE[b]	RMSE[c]	|Le%|[d]
AUC				
Average of 10 neural networks	−0.00270	0.00284	0.05326	20.74
Random learning set	0.00629	0.00323	0.05683	25.14
c_{max}				
Average of 10 neural networks	−0.00981	0.00889	0.09422	35.22
Random learning set	−0.00566	0.00686	0.08281	33.59
t_{max}				
Average of 10 neural networks	−0.01251	0.00197	0.04436	27.58
Random learning set	−0.00950	0.00190	0.04400	27.94

[a] ME: mean error.
[b] MSE: mean squared error.
[c] RMSE: root mean squared error.
[d] |Le%|: absolute learning error percent.

higher output values compared with the measured values. Learning performance was the best in neural networks for AUC prediction. Some values of |Le%| were higher and reached 148.09% for AUC, 162.74% for c_{max}, and 169.45% for t_{max} neural networks. The validation of networks prediction is shown in Table II. Few individual cases occur with each parameter where the network yields much higher |Pe%|. The maximum |Pe%| was 232.85% for AUC, 253.23% for c_{max}, and 290.23% for t_{max}. These cases appear to be linked with the outlying pharmacokinetic response in a particular individual. For each test set, the F-test for comparison of the variances of measured and predicted values was performed. For AUC, 7 of the 10 test sets had insignificant differences in the variance of measured and predicted output values, and the difference in average coefficient of variation for measured and predicted values was 7.55%. In 8 of the 10 test sets the difference between the average of measured and predicted values was insignificant. For c_{max}, all 10 test sets showed significant differences in the variance of measured and predicted output values and the difference in average coefficient of variation for measured and predicted values was 36.71%. Neural networks for c_{max} were unable to perceive a variability of the measured values. In 6 of the 10 test sets the difference between the average of measured and predicted values was significant. There were insignificant differences between the average of measured and predicted values of t_{max} in 8 of the 10 test sets. The difference in the average coefficient of variation for measured and predicted values was 20.06%, which indicates these networks had a low ability to perceive the variance of t_{max}. In Fig. 1, measured and predicted values of AUC are presented for one typical test set.

Five individuals, who were randomly excluded from the database before its division into 10 operational data sets, were added to each test set. The average absolute prediction error percent when a prediction was performed on these individuals was: 25.63% for AUC (average for all 10 test sets with a minimum 15.33% and a maximum 47.11%), 40.23% for c_{max} (average for all 10 test sets with a minimum 35.44% and a maximum 42.49%), and 32.25% for t_{max} (average for all 10 test sets with a minimum 30.32% and a maximum 36.20%). The differences between a test set with the maximum prediction error and a test set with the minimum prediction error reveals, that despite the relatively large size of the entire database (n = 98 subjects and 312 administrations), prediction performance still depends on the individuals who are included in the training matrix.

A sensitivity analysis was performed on the basis of neural models for the prediction of pharmacokinetic parameters. It demonstrated that, for verapamil, the four most influential variables assigned to input neurons were: total protein concentration, concentration at t_0; AST levels and heart-rate for AUC; AST levels, total protein concentration, concentration at t_0, and ALT levels for c_{max}, and the presence of food, blood pressure, constitution and AST levels for t_{max}. Less influential variables were: formulation, period, and the presence of food for AUC; sequence, period and formulation for c_{max}, and serum concentration of alkaline-phosphatase, sequence and concentration at t_0 for t_{max}.

Input variables were also sorted with regard to the parameter u. The most influential input variables have higher values of the parameter u. These are: concentration at t_0, presence of food, AST levels, name of the individual, ALT levels and serum bilirubine for AUC; concentration at t_0, AST levels, presence of food, name of the individual, ALT levels and serum concentration of alkaline-phosphatase for c_{max} and presence of food, name of the individual, AST levels, constitution, concentration at t_0 and ALT levels for t_{max}.

Four linear regression models were built for AUC and c_{max}, and two for t_{max}. Values of AUC and c_{max} were predicted from concentration at t_0, serum total proteins, AST levels, and serum calcium. The differences in average coefficient of variation for measured and predicted values were: 28.52% (serum total proteins), 22.16% (concentration at t_0), 29.57% (AST levels), 30.68% (serum calcium) for AUC models and 45.31% (serum total proteins), 43.41% (concentration at t_0), 44.99% (AST levels), and 49.38% (serum calcium) for c_{max} models. Values of t_{max} were predicted from concentration at t_0 and presence of food. The differences in the average coefficient of variation for measured and predicted values of t_{max} were: 22.52% (presence of food) and 35.36% (concentration at t_0).

DISCUSSION

The statistical analysis of the bioequivalence study results includes the determination of pharmacokinetic parameters. The variability sources of these parameters are usually analyzed

Table II. Validation of Networks Prediction Performance

	ME	MSE	RMSE	|Pe %|[a]	CV_m[b] %	CV_p[c] %
AUC						
Average of 10 neural networks	−0.00365	0.00553	0.07343	30.54	39.53	31.98
Random learning set	0.02716	0.00964	0.09816	39.57	49.42	30.35
c_{max}						
Average of 10 neural networks	−0.01120	0.01060	0.10190	39.56	54.14	17.43
Random learning set	0.03877	0.02287	0.15123	53.54	63.81	20.66
t_{max}						
Average of 10 neural networks	−0.00885	0.0024	0.0486	30.74	43.78	23.72
Random learning set	−0.0102	0.0018	0.0430	28.44	39.06	25.35

[a] |*Pe%*|: absolute prediction error percent.
[b] CV_m: coefficient of variation for measured values.
[c] CV_p: coefficient of variation for predicted values.

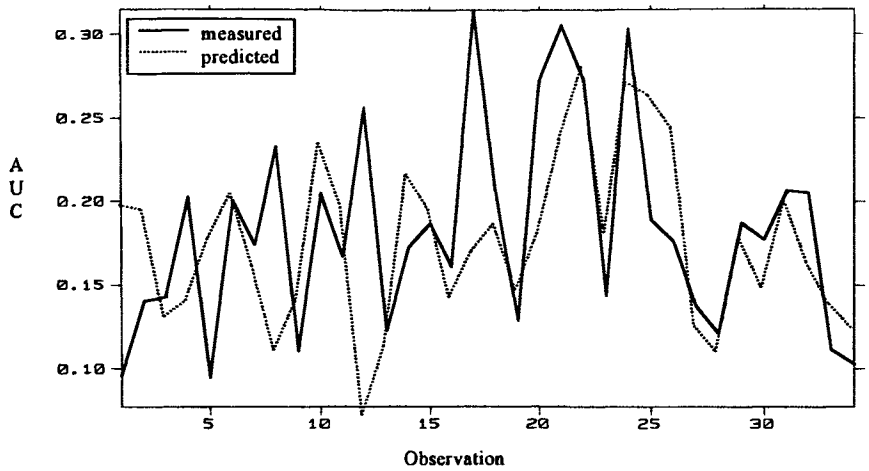

Fig. 1. Measured and predicted values of AUC. The test set $|Pe\ \%|$ is 28.19%.

with the aid of ANOVA and linear models. The concept of the analysis of variance is to study the variability in the observed data by partitioning the total sum of squares of the observations into components of fixed effects and random errors. For the standard 2 × 2 crossover design, we would partition the total sum of squares into components for the carry-over effects, the period effect, the direct drug effect, and the error (1).

We tried to define variability sources more precisely and better explain intraindividual and interindividual variability for verapamil. Besides variables of the study design (sequence, period, formulation, food), we used another 15 variables. We thought they could contribute to the total variability. Biochemical parameters that were used as input variables were measured once before the clinical part of the study. This may contribute to the increase of prediction error of pharmacokinetic parameters because we were unable to note changes in the biochemical parameters that could occur between applications of two formulations. If neural networks would be learned with repeated dose data, then models could perceive intraindividual changes in the PK parameters. In a standard 2 × 2 crossover design models can perceive only interindividual changes in the PK parameters. Therefore by measuring biochemical parameters during the study, intraindividual changes in defined input variables could be detected and models could predict intraindividual PK parameter changes.

Neural networks were used as a tool because no presumptions are needed for the evaluation of results and because of very noisy data. The average prediction error of pharmacokinetic parameters is from 30.54% for AUC to 39.56% for c_{max}. The error seems to be high, but verapamil is a highly variable drug with an intraindividual coefficient of variation of about 23% for AUC and 29% for c_{max} (17). A prediction of the same pharmacokinetic parameter on five individuals was different among 10 neural networks. We concluded that the results still depend on the individuals who are included in the training sets and that an increase in the training matrix data could improve that prediction. Neural networks also gave the best results with test data that were in the range of training matrix data. All outliers (namely individuals whose observed pharmacokinetic parameters were outside the learning range) had a higher prediction error than other individuals.

A sensitivity analysis was performed on the basis of neural models. With the sensitivity analysis all 19 input variables were sorted according to influence on output variable changes. The sensitivity analysis results show drug concentration at time t_0 (or before application of the dose after which pharmacokinetic parameters were determined) is important for AUC and c_{max}. This is a consequence of database properties, which included data from single and multiple bioequivalence study designs and confirms the accuracy of the sensitivity analysis results. The bioavailability of verapamil is 20–35% and protein binding about 90%. It is known the protein binding of drugs is related to AUC and c_{max} and the sensitivity analysis results show a high influence of verapamil plasma protein concentration on AUC and c_{max}. The serum concentration of liver enzymes was determined as influential for AUC and c_{max}. This could be connected with low bioavailability and the extensive first-pass effect of verapamil. The presence of food was determined as the most influential parameter for t_{max} variability. This could be explained by the influence of food on the rate of drug absorption.

An impact of input variable on output variable values depends on its sensitivity and variability in the population of individuals. Therefore a new parameter (u) was calculated. Higher values of the parameter u signify a more influential input variable except in cases of nominal input variables, whose defined numeric values were often extreme and the variability of those variables cannot be numerically interpreted. The influence of nominal variables cannot be precisely determined in this way. The results of this ranging show that serum concentration of liver enzymes and concentration at time t_0 belong to the most influential variables as in the sensitivity analysis results.

All linear regression models were worse perceiving pharmacokinetic parameter variability compared to the neural models.

This study demonstrated backpropagation neural networks are a useful tool for the prediction of verapamil pharmacokinetic parameters. Neural models could also be used for the determination of variability sources. Developed neural models are useful only for the prediction of verapamil parameters. More investigations with a developed methodology should be made. Neural networks should be learned with the database of other substances, i.e., other calcium antagonists, and the results of input

factor influence should be compared. Because of the very variable data, widening the database could give better results or a lower prediction error of neural networks. The developed methodology offers an opportunity for selecting individuals for bioequivalence studies whose properties would provide a smaller variability of pharmacokinetic parameters and an increased power of the study. The most influential input factors should be scrutinized in the protocol before or during the study. In this way a higher accuracy of bioequivalence study outcome could be obtained (a high intraindividual variability of pharmacokinetic parameters could produce the wrong outcome of the bioequivalence study) while offering the possibility of using fewer subjects.

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