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*Research Paper*

# Population Pharmacokinetic Data Analysis of Cilobradine, an $I_f$ Channel Blocker

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***Purpose.*** To evaluate the population pharmacokinetic characteristics of cilobradine including a covariate analysis based on six phase I trials and to assess the predictive performance of the model developed.
***Methods.*** Single or multiple doses of cilobradine were administered as solution, capsule or infusion. Two thousand, seven hundred and thirty-three plasma samples (*development* data set) were used for model development in NONMEM. Model evaluation was performed using also an *external* data set.
***Results.*** Data were best described by a linear three-compartment model. Typical $V_{ss}$ was large (~100 l) and CL was 21.5 l/h. Covariate analysis revealed a statistically significant but clinically irrelevant relation between KA and dose. Inter-individual variability was moderate (15–46%); imprecision of estimates was generally low. The final model was successfully applied to the *external* data set revealing its robustness and general applicability. Its final estimates resembled those of the *development* data set except for the covariate relation not being supported. When excluding the covariate relation, all observations were well predicted.
***Conclusion.*** A robust population PK model has been developed for cilobradine predicting plasma concentrations from a different study design well. Therefore, the model can serve as a tool to simulate and evaluate different dosing regimens for further clinical trials.

**KEY WORDS:** cilobradine; $I_f$ channel blocker; NONMEM; population pharmacokinetics.

## INTRODUCTION

Ischemic heart disease is characterised by an imbalance between myocardial oxygen supply and demand resulting in ischemic pain, myocardial dysfunction, or tissue necrosis. As heart rate is a major determinant of myocardial energy demand drug-induced bradycardia is expected to reduce myocardial oxygen consumption (1,2). Heart rate reduction at rest and during exercise has been considered as an important mechanism for the antianginal and antiischemic effects of β-adrenergic blocking agents and some calcium channel blockers (3,4). In spite of their known clinical efficacy in the treatment of ischemic heart disease, certain side effects such as negative inotropism and hypotensive effects impairing ischemic status may limit their use (5). The so-called specific bradycardic agents represent a new class of compounds developed to selectively reduce heart rate with little if any effect on other cardiovascular parameters (6–8). They reversibly block the cardiac pacemaker current (also called the "funny current," $I_f$) passing through the hyperpolarisation-activated cyclic nucleotide-gated (HCN) channel ($I_f$ channel) (9), resulting in a reduced slope of the diastolic depolarisation of the pacemaking cells of the sinoatrial node. Thus, due to heart rate reduction they not only decrease oxygen demand but also increase oxygen supply to the ischemic myocardium via an increase in the diastolic blood flow (5,10). Alinidin and falipamil, derivatives of clonidine and verapamil, respectively, were the first compounds of this class followed by zatebradine (11,12), a more potent, longer acting, and more specific benzazepinone derivative of falipamil (13). Due to certain side effects including hypotension, negative inotropism or visual disorder (14–16), or insufficient efficacy the clinical development of these drugs was terminated (15,16). The search for more specific bradycardic agents led to the development of the benzazepinone derivatives ivabradine and cilobradine. Ivabradine (Procoralan®) was approved in October 2005 by the European Agency for the Evaluation of Medicinal Products (EMEA) for the treatment of chronic stable angina pectoris. Favourable effects of cilobradine were shown in various *in vivo* models (9,17). Therefore, cilobradine was evaluated in several phase I clinical trials and might be beneficial in the treatment of cardiovascular diseases, e.g. ischemia.

The present analysis characterises for the first time the pharmacokinetics (PK) of cilobradine as a compound of a new

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drug class in healthy volunteers by the population analysis approach. In detail, the objectives of the present analysis were (1) to thoroughly describe the PK profile and variability of cilobradine based on data of several clinical phase I trials with a broad dose range and different formulations, (2) to identify covariates influencing the PK of cilobradine, (3) to evaluate the developed population PK model and its predictive performance based on the data used for model development and additionally with data from a different phase I trial.

## METHODS

### Study Design

Model development was performed using data of six phase I trials (*studies A–F*, development data set). Model evaluation was conducted using also data from another phase I trial (*study G*, external data set). The study characteristics and sampling schedules of the data sets are given in Table I.

All studies were performed at the Human Pharmacology Centre of Boehringer Ingelheim (Biberach, Germany). Except for *study C*, all studies were performed with a randomised, double-blind (*study G*: partly double-blind), placebo-controlled, parallel group design. S*tudy C* was a randomised, open three-way cross-over trial in which bioavailability of cilobradine was assessed. All three formulations were investigated in three successive periods with a wash-out period between treatments of seven days.

### Analytical Methods

Cilobradine in plasma samples was quantified by high-performance liquid chromatography (HPLC) and enzyme-linked-immunosorbent-assay (ELISA). In brief, the HPLC assay was a reversed phase method with fluorescence detection (excitation at 280 nm, emission at 320 nm). The lower limit of quantification (LLOQ) was 1.5 ng/ml. Thus, plasma samples especially at late sampling time points were quantified by a competitive ELISA with a LLOQ of 0.1 ng/ml. The assays were validated according to international criteria (18).

### PK Data Analysis

For model development, all data of the development data set were analysed simultaneously using the non-linear mixed effects modelling approach with the software NONMEM (19),

**Table I.** Study Characteristics and Sampling Schedule of the Development (Studies A–F) and External (Study G) Data Set

| Study | Subjects[a] | Observations | Formulations | Doses [mg] (subjects) | Dosing | Sampling Schedules[b] |
|---|---|---|---|---|---|---|
| A | 42 | 344 | p.o. solution | 1.25(6), 2.5(6), 5(6), 10(6), 20(6), 30(6), 40(6) | SD[c] | 1.25–40 mg: pre-dose, 0.5, 1, 2, 4, 8, 12, 24, 32 h p.a.[d]<br>10 mg additionally: 10, 25, 45 min, 1.5, 3, 5, 6, 10 h p.a. |
| B | 23 | 264 | i.v. infusion | 2.5(6), 5(6), 10(6), 15(5) | SD | 2.5–15 mg: pre-dose, 18, 21, 30 min, 1, 2, 4, 8, 12, 24, 32 h p.a.<br>10 mg additionally: 10, 25, 45 min, 1.5, 3, 5, 6, 10 h p.a. |
| C | 18[e] | 903 | p.o. solution<br>p.o. capsule<br>i.v. infusion | 10(18)<br>10(18)<br>10(17) | SD × 3 (cross-over) | i.v.: pre-dose, 10, 18, 21, 25, 30, 45 min, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 24, 32 h p.a.<br>p.o.: pre-dose, 15, 30, 45 min, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 10, 12, 24, 32 h p.a. |
| D | 30 | 882 | p.o. capsule | 5(12), 10(12), 20(6) | MD[f] (qd, 7 d) | day 1 and 7: pre-dose, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 9, 10.5, 12 h p.a.<br>day 2–6: pre-dose<br>24, 33, 48 h after the last dose |
| E | 24 | 236 | p.o. capsule | 10(12), 20(12) | SD | pre-dose, 0.25, 0.5, 1, 2, 3, 6, 12, 24, 32, 48 h p.a. |
| F | 25 | 104 | p.o. capsule | 0.6(6), 1.25(8), 2.5(11) | MD (qd, 15 d) | day 1, 2, 3, 8 and 15: pre-dose<br>24, 48 h after the last dose |
| G | 76 | 1,713 | p.o. solution | 0.25(12), 0.5(12), 1(18), 2(16), 5(18) | MD (qd, 15 d) | day 1 and 14: 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 h p.a.<br>24, 48, 72, 144 h after the last dose |

[a] Analysed subjects treated with cilobradine
[b] Protocol time points
[c] Single dosing
[d] Post administrationem
[e] Corresponding to 53 different plasma profiles
[f] Multiple dosing

version V, level 1.1. Due to the rich data situation, the FOCE INTERACTION estimation method was used. The parameterisation of all PK models investigated was in terms of clearances and distribution volumes. First, the basic population model including the structural and pharmacostatistical submodel was specified. The structural submodel was determined by fitting different one and multiple (two-, three- or four-) compartment models and by investigating the absorption process of cilobradine testing first-order, zero-order and mixed-order (combination of zero- and first-order) input. The inter-individual variability as part of the pharmacostatistical submodel was modelled with an exponential random effects term

$$P_{ki} = \theta_k \cdot e^{\eta_{ki}}$$

$P_{ki}$ represents the value of the PK parameter $k$ for the $i$th individual. $\theta_k$ symbolises the typical population value of $k$, and $\eta_{ki}$ the deviation of $P_{ki}$ from $\theta_k$. $\eta_{ki}$ was assumed to be symmetrically distributed in the population, with a mean of zero, and an estimated variance $\omega_k^2$ . The $\omega_k^2$ of the model parameters are the diagonal elements of the variance–covariance matrix $\mathbf{\Omega}$. The off-diagonal elements of $\mathbf{\Omega}$, representing the covariance between the diagonal elements, were also explored on stage of the final covariate model, see below.

If parameters showed random variability within individuals between study occasions (inter-occasion variability) this variability was also examined:

$$P_{kiq} = \theta_k \cdot e^{\eta_{ki}+\kappa_{kiq}}$$

$P_{ki}$ at occasion $q$ differs from the typical individual $P_{ki}$ by an additional random effect, $\kappa_{ki}$ (zero mean, variance $\pi^2$), which accounted for the between-occasion variability.

Residual variability as another part of the pharmacostatistical submodel reflecting the difference between the observed and model-predicted concentrations is represented by the random variable $\varepsilon_{ij}$ (zero mean, variance $\sigma^2$) and was modelled using additive, proportional or combined error models.

Once the basic model provided an adequate description of the PK profile and variability of cilobradine, the demographic and laboratory patient characteristics listed in Table II and study characteristics such as dose, formulation, analytical assay and study no. were explored for significant covariate effects to explain parts of the initial inter-individual variability.

The potential covariates were initially screened using the stepwise generalised additive modelling (GAM) approach implemented in the software Xpose® version 3.10 (20,21). In addition, parameter covariate relations were investigated from plots of individual Bayesian posthoc estimates of the parameters *versus* covariates (22). Preselected covariates were further assessed for significance in NONMEM by forward inclusion and backward exclusion techniques (23). Covariates were incorporated one at a time until the full covariate model was obtained ($p = 0.05$, $df = 1$). Appropriate functions (linear and non-linear) for each continuous covariate describing the parameter covariate relation were investigated, e.g.:

$$\theta_{\mathrm{PK,Cov}} = \theta_{\mathrm{PK}} \cdot \left(1 + \theta_{\mathrm{Covariate,PK}} \cdot (\mathrm{Covariate} - \mathrm{Covariate_{med}})\right)$$

$\theta_{\mathrm{PK,\ Cov}}$ represents the typical value of the PK parameter for a certain covariate value, $\theta_{\mathrm{PK}}$ the estimated typical value of the PK parameter at the median value of the continuous covariate ($\mathrm{Covariate_{med}}$), and $\theta_{\mathrm{Covariate,\ PK}}$ the estimated effect of the covariate as the percentage change of $\theta_{\mathrm{PK}}$ per one covariate unit deviation from $\mathrm{Covariate_{med}}$. If the graphical analysis indicated a non-linear parameter covariate relation, non-linear functions were evaluated, e.g. the following positive saturation function:

$$\theta_{\mathrm{PK,Cov}} = \mathrm{PK_{max}} \cdot \mathrm{Covariate}/(\mathrm{Covariate_{PK_{50}}} + \mathrm{Covariate})$$

$\mathrm{PK_{max}}$ is the maximum value of the PK parameter, $\mathrm{Covariate_{PK_{50}}}$ is the value of the covariate at which the PK parameter is half of its maximum value.

All parameter covariate relations from the full covariate model were re-tested by stepwise backward deletion applying a stricter statistical criterion ($p = 0.001$, $df = 1$). The final covariate model was obtained when deletion of each covariate was significant. For building the final population PK model, the refinement of all components of the final covariate model was performed, e.g. covariance between the diagonal elements of the $\mathbf{\Omega}$ matrix was investigated.

Model selection on each stage of model development was based on the precision of parameter estimates, goodness-of-fit plots as well as changes in the objective function value (OFV). Precision of parameters was expressed as the relative standard error (RSE) of the parameters. The OFV is approximately proportional to −2*log likelihood of the data, under the assumption that the model is the true model and that the errors are symmetrically distributed (24,25). The differences in OFV (ΔOFV) between two nested models are approximately $\chi^2$ distributed, with $df$ being equal to the number of differing fixed effect parameters. Thus, the improvement in model fit by the inclusion (or deletion) of a model parameter can be assigned a significance level. ΔOFVs of 3.84 and 10.83 correspond to nominal $p$ values of 0.05 and 0.001 ($df = 1$), respectively. During model development for nested models the $p$ values of 0.05 (base model development and covariate forward inclusion) and 0.001 (covariate backward

**Table II.** Demographic and Laboratory Characteristics of the Development Data Set

| Characteristics | Median | Range |
|---|---|---|
| Demographic | | |
| Age (years) | 29 | (18–54) |
| Height (cm) | 180 | (164–192) |
| Weight (kg) | 76 | (57–102) |
| Laboratory | | |
| Heart rate at rest ($min^{-1}$) | 64 | (50–93) |
| $BP_{syst}$ at rest (mmHg) | 131 | (105–160) |
| $BP_{diast}$ at rest (mmHg) | 75 | (51–90) |
| Creatinine clearance (ml/min) | 114.8 | (76.4–175.2) |
| AST (U/L) | 10.9 | (5.0–24.0) |
| ALT (U/L) | 11.0 | (2.0–25.6) |
| GGT (U/L) | 13.0 | (4.4–52.6) |
| AP (U/L) | 104.4 | (45.0–175.2) |
| LDH (U/L) | 129.0 | (93.4–208.0) |

*$BP_{syst}$* Systolic blood pressure, *$BP_{diast}$* diastolic blood pressure, *AST* aspartate aminotranferase, *ALT* alanine aminotranferase, *GGT* gamma-glutamyl transferase, *AP* alkaline phosphatase, *LDH* lactate dehydrogenase

elimination) were assigned to including and deleting a model parameter, respectively.

The predictive performance of the final population PK model was evaluated using the data of the development data set (*internal evaluation*) and of the external data set (*external evaluation*). Evaluation was performed according to the FDA guideline (26). For the *internal evaluation* the development data set was reduced to the single dose data of all 10 mg dose groups where most data was available and that was studied in one third of the subjects and in all studies excluding study F. In addition, the reduction achieved reasonable run times for the simulations. One thousand new data sets with the same study design and subject characteristics of the reduced development data set were simulated by fixing the model parameters of the final population PK model. The interval including 90% of the simulated concentrations (= 90% prediction interval) was constructed computing the 5th and 95th percentiles at each time point and the simulated median concentration-time profile corresponded to the 50th percentile. The observed data of the reduced development data set were then compared with the 90% prediction interval and the simulated median concentration-time profile. The median (MDPE) and median absolute (MDAPE) prediction errors were computed to access the accuracy and precision for predicting the data of the reduced development data set by the final population PK model (27,28). Prediction error (PE) for each observation was defined as

$$\text{PE, \%} = \frac{C_{\text{sim}} - C_{\text{obs}}}{C_{\text{obs}}} \cdot 100$$

$C_{\text{sim}}$ is the simulated and $C_{\text{obs}}$ the observed concentration. For *external evaluation*, the external data set was used both for simulation and for estimation. Five hundred new data sets with the same characteristics as the external data set were simulated to determine the 90% prediction interval, the median concentration-time profile, MDPE and MDAPE. In addition, the fixed and random effects parameters of the final population PK model were estimated using the external data set to compare them with those estimates based on the development data set.

## RESULTS

### Development of the Basic Model

Disposition characteristics of cilobradine were best described by a three-compartment model with first-order absorption and elimination. Compared to a one- or two-compartment model, the three-compartment model led to a reduction in OFV of more than 1,200 points ($p > 0.05$). The separate estimation of the absolute bioavailability for p.o. solution ($F1_{sol}$) and for p.o. capsule ($F1_{cps}$) data resulted in a significant drop in OFV of 30 points. In addition, the incorporation of a lag time for the capsule ($Tlag_{cps}$) provided a significant drop of OFV of 80 points. The three-compartment model predicted the individual observed concentrations well.

However when some profiles of subjects that displayed higher than average profiles especially around the time of the maximum concentration but did not display any special characteristics (e.g. covariate values) were excluded model parameters could not successfully be estimated. When evaluating the oral data alone they also could be well described by a three-compartment model, however KA was much smaller. Hence, in order to obtain a model that was tolerant to exclusion of values and described all data well the parameters V2, V3 and Q3 were separately estimated for i.v. or p.o. data. A schematic plot of the structural model is given in Fig. 1.

Inter-individual variability was associated with total plasma clearance CL, absolute bioavailabilities $F1_{sol}$ and $F1_{cps}$, absorption rate constant KA and apparent volume of distribution of the central compartment for i.v. data $V2_{iv}$. The estimation of a single inter-individual variability on F1 leading to one $\eta_{F1}$ value per subject did not result in an optimal fit in the cross-over *study C*. In addition to the different typical values of F1 for the p.o. solution and the p.o. capsule the variability in F1 did not seem to be subject—but within one subject formulation—dependent. Due to the described phenomenon the inter-individual variability of F1 for the p.o. solution and p.o. capsule was finally coded as SAME BLOCK provided by NONMEM (29). Thus, a common variance $\omega^2$ of the random effects parameter $\eta_{F1_{sol}}$ and $\eta_{F1_{cps}}$ was estimated but different individual values of F1 for the p.o. solution and p.o. capsule per subject in the cross-over *study C* were possible providing for each subject a much better fit. The same result was obtained when estimating two different variances for the absolute bioavailability of the p.o. solution and p.o. capsule. However, this model was not superior to the SAME BLOCK code but included one additional parameter to be estimated and was therefore not further pursued. Based on the exponential variability model associated with F1, even for the subjects who did not receive an i.v. dose the individual empirical Bayes F1 estimates were within the plausible range of 0 to 1. The establishment of an inter-individual variability of V2 was successful when restricting it to the i.v. data resulting in a better individual fit. The residual error was best modelled with a proportional error model. Accounting for the two different analytical methods (HPLC, ELISA: for lower concentrations) by including different additive error terms for lower and higher concentrations, respectively, did not lead to any improvements. Table III (left panel) shows the parameter estimates obtained from the basic population model.

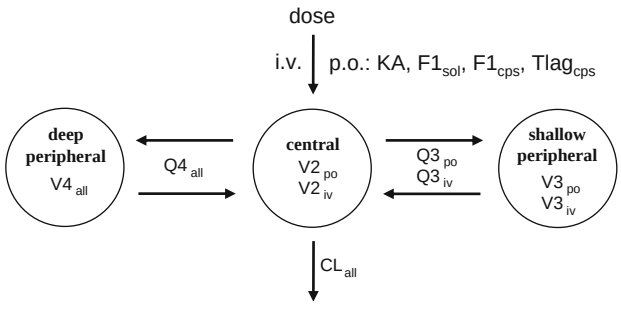



**Fig. 1.** Schematic structural model of cilobradine. *KA* Absorption rate constant; $F1_{sol}$ and $F1_{cps}$ absolute bioavailability of the p.o. solution and the p.o. capsule, respectively; $Tlag_{cps}$ lag time of the p.o. capsule; *V2*, *V3* and *V4* apparent volumes of distribution of the central, shallow and deep peripheral compartments, respectively; *CL* total plasma clearance; *Q3* and *Q4* inter-compartmental clearances corresponding to the shallow and deep peripheral compartments, respectively; estimation of parameter $_{all}$, $_{po}$, $_{iv}$ based on all, p.o. and i.v. data, respectively.

**Table III.** Population Pharmacokinetic Estimates of Cilobradine Obtained from the Basic and Final Model

| Model Parameter | Basic Model (internal data) | | Final Model (internal data) | | Final Model (external data) | |
|---|---|---|---|---|---|---|
| | Population Estimate | RSE[a], % | Population Estimate | RSE[a], % | Population Estimate | RSE[a], % |
| CL (l/h) | 20.9 | 6 | 21.5 | 6 | 18.7 | 4 |
| V2 (l) | 9.28 (p.o.)/25.1 (i.v.) | 25/8 | 9.10 (p.o.)/24.5(i.v.) | 24/8 | 9.03 | 9 |
| V3 (l) | 34.1 (p.o.)/53.0 (i.v.) | 13/7 | 33.8 (p.o.)/52.9(i.v.) | 13/7 | 52.6 | 6 |
| V4 (l) | 51.6 | 12 | 52.9 | 12 | 85.1 | 8 |
| Q3 (l/h) | 6.73 (p.o.)/99.8 (i.v.) | 16/6 | 6.61 (p.o.)/99.8 (i.v.) | 16/6 | 8.07 | 14 |
| Q4 (l/h) | 1.32 | 7 | 1.34 | 7 | 0.997 | 18 |
| $KA_{max}$ ($h^{-1}$) | 0.384[b] | 5 | 0.430 | 5 | 0.408[b] | 5 |
| $Dose_{KA_{50}}$ (mg) | n.a. | n.a. | 1.00 | 15 | n.a.[c] | n.a |
| $Tlag_{cps}$ (h) | 0.152 | 56 | 0.154 | 52 | n.a. | n.a. |
| F1 (%) | 33 (sol)/44 (cps) | 6/7 | 34 (sol)/43 (cps) | 6/7 | 34[d] | n.a. |
| Inter-individual variability | | | | | | |
| $\omega_{CL}$ (%CV) | 27 | 15 | 25 | 15 | 28 | 19 |
| $\omega_{F1_{sol/cps}}$ (%CV) | 35[e] | 17 | 34[e] | 16 | 39 | 15 |
| $\omega_{KA}$ (%CV) | 17 | 25 | 15 | 26 | 15 | 26 |
| $\omega_{V2_{iv}}$ (%CV) | 49 | 22 | 46 | 19 | n.a. | n.a. |
| $Covariance_{CL/V2_{iv}}$ | n.a. | n.a. | 0.0306 | 28 | n.a. | n.a. |
| Residual error | | | | | | |
| $\sigma_{proportional}$ (%CV) | 26 | 7 | 26 | 7 | 20 | 7 |

[a] Relative standard error (standard error divided by population estimate *100; for the random effects parameters RSE is related to the corresponding variance scale)
[b] Value of KA
[c] When the covariate relationship was included $Dose_{KA_{50}}$ was $< 10^{-10}$,with a RSE of $> 10^{7}$
[d] Fixed parameter
[e] Same variance of F1 for p.o. solution and p.o. capsule coded as SAME BLOCK; *n.a.* not applicable

## Covariate Analysis and Refinement of the Final Covariate Model

Twenty covariate relations were found in the GAM analysis or by graphical inspection: CL ~ study, dose, age, ALT (alanine aminotranferase), GGT (gamma-glutamyl transferase) and AP (alkaline phosphatase); $F1_{sol}$ ~ AP and study; $F1_{cps}$ ~ AP, study, height and age; KA ~ study, dose, age, systolic blood pressure at rest and heart rate at rest; $V2_{iv}$ ~ study, AP and ALT. Due to the facts that (a) the basic population PK model already considered possible differences arising from the different studies (e.g. formulation and route of administration effects) and (b) graphical inspections displayed only minor relations, the influence of the covariate 'study' on the PK parameter was not further investigated. Besides, individual study groups were compared using the two-sided Kruskal–Wallis rank sum test. No statistically significant ($p < 0.05$) differences were observed for age, height, or weight between studies, i.e. also not for study A (p.o.) and B (i.v.; both dose-escalation trials with similar study design and rich

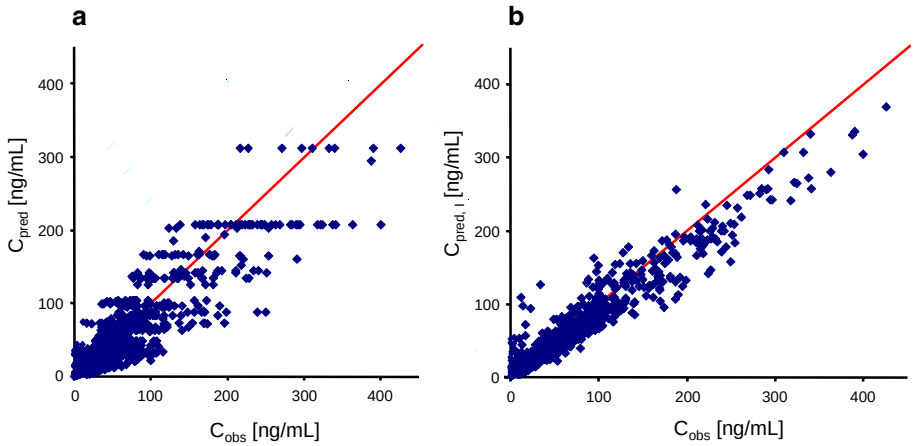



**Fig. 2.** **a** Population predicted concentrations ($C_{pred}$) and **b** individual predicted concentrations ($C_{pred, I}$) *vs* observed concentrations; the *red line* represents the line of unity; $N = 2{,}733$.

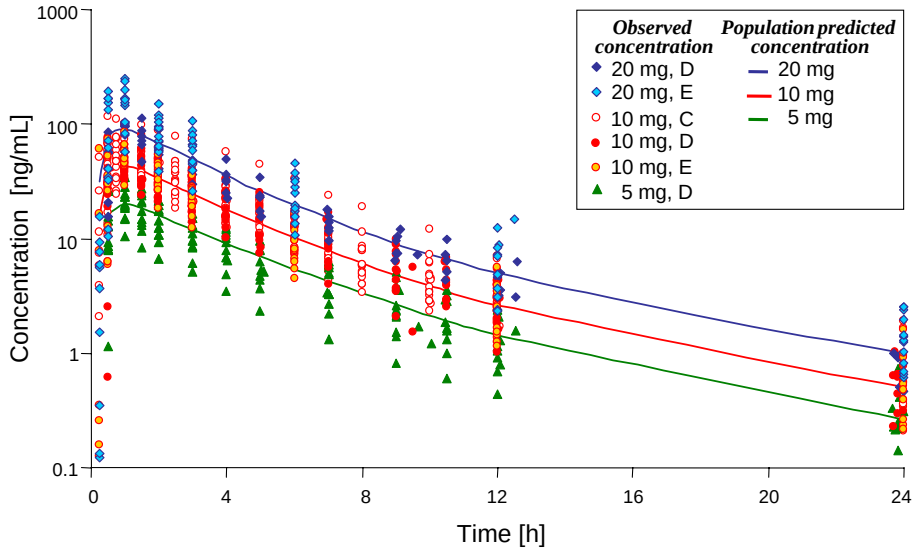



**Fig. 3.** Population predicted (*lines*) and observed (*symbols*) concentration-time profile after p.o. administration of a 5, 10 and 20 mg cilobradine capsule; *C*, *D* and *E* correspond to the respective studies.

sampling schedule, see Table I). The forward inclusion and backward elimination procedure of the covariates led to the final covariate model with one remaining parameter covariate relation between KA and dose. The relation was best described by a positive saturation function compared to other investigated functions (e.g. linear function).

The visual graphical inspection of the random effects parameters of the final covariate model indicated a significant covariance between $\omega^2_{CL}$ and $\omega^2_{V2_{iv}}$ as a positive correlation. The incorporation of the covariance in the final covariate model resulted in a significant drop of OFV of almost 8 points. The corresponding coefficient of correlation was 0.750. Thus, the mentioned covariance remained in the final covariate model representing the final population PK model.

## Final Population PK Model

Table III (middle panel) lists all parameter estimates obtained from the final population PK model using the FOCE INTERACTION method. RSEs of the fixed and random effects parameters were generally small between 5 and 28%, which suggests that these parameters were estimated with high precision, with the exception of $Tlag_{cps}$ (52%). The goodness-of-fit plots obtained from the final population PK model are shown in Fig. 2.

The absence of distinct systemic deviations in the goodness-of-fit plots additionally indicates that the selected model described the data sufficiently well. As an example, Fig. 3 shows the typical population model predicted plasma concentration *vs* time profiles as a function of the dose of cilobradine (5, 10 and 20 mg) given orally as capsule, together with the observed data of the studies C, D and E in a semi-logarithmic plot. Overall, the predicted concentrations well reflected the main tendency of the observed concentrations in each dose group with a slight trend to underpredict high observed concentrations.

The parameter covariate relation between KA and dose best described by a positive saturation function resulted in a $KA_{max}$ of 0.429 $h^{-1}$. This value was almost reached at the dose of 5 mg. The dose at 0.5 $KA_{max}$ ($Dose_{KA_{50}}$) was 0.99 mg, i.e. the relation was primarily acting in the low dose range. By incorporating the parameter covariate relation only a few observed concentrations could be better described.

## Evaluation of the Final Population PK Model

### *Internal Evaluation*

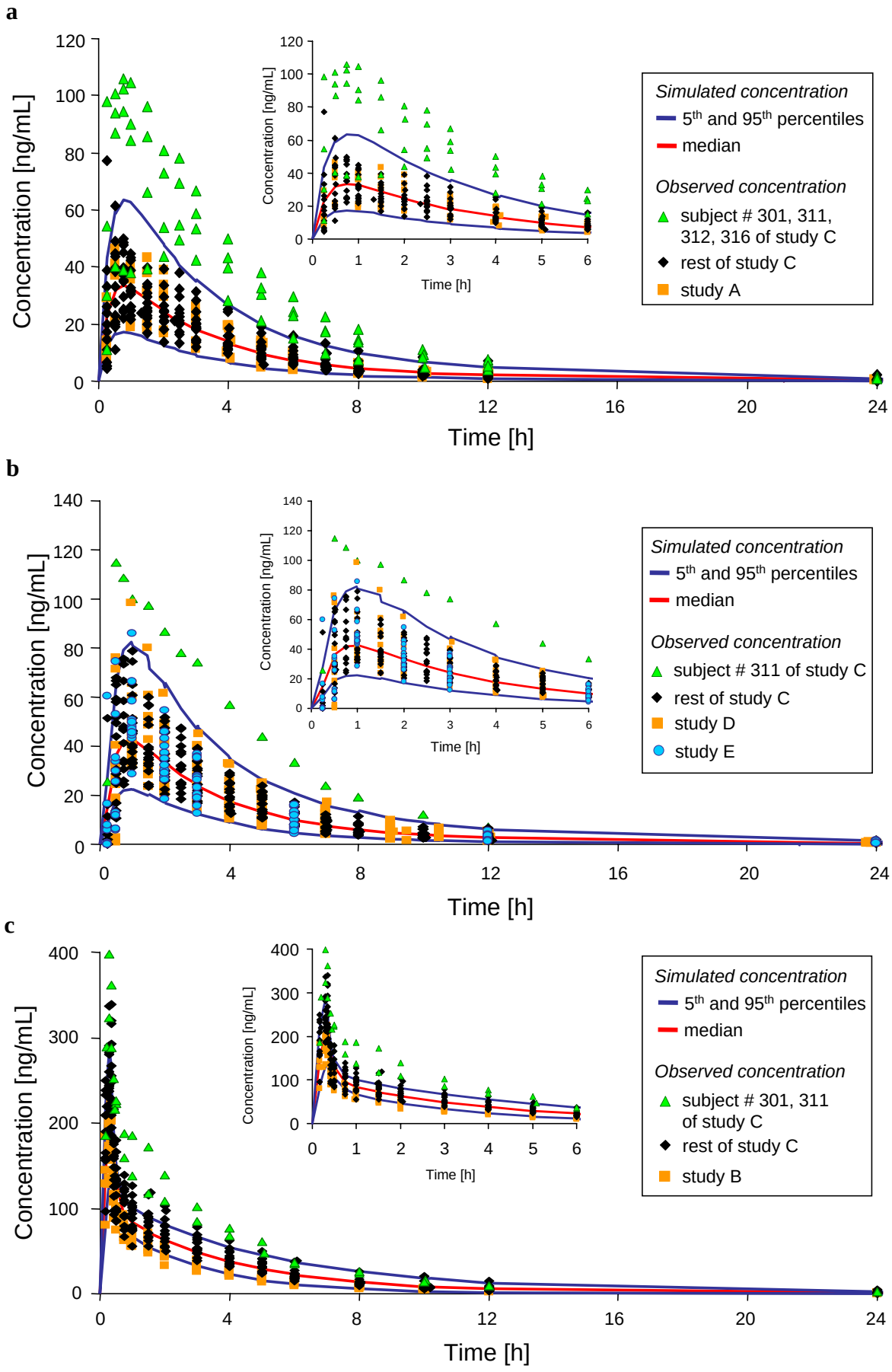
The 90% prediction interval adequately encompassed the observed concentration-time data of the selected development data set of the p.o. solution and p.o. capsule (Fig. 4a, b).

No more than 10% of the observations were outside the range. These mainly originated from single individuals from *study C* (green triangles). In both cases the simulated median concentration-time profile showed a slight trend to underprediction. Fig. 4c represents the predictive performance of the model for the observations of the i.v. administration. The 90% prediction interval covered only about 77% of the observations. The concentrations of study B (orange symbols) were tendentiously overpredicted maybe due to one or more covariates which have not yet been identified. The values of MDPE and MDAPE were −4 and 38%, respectively. The negative value of MDPE confirmed the slight underpredicting tendency of the model.

### *External Evaluation*

The estimates of the final population PK model based on the external data set were very similar to those obtained by the development data set except for the parameter $Dose_{KA_{50}}$. This parameter was stated to be $<10^{-10}$ mg with a huge RSE

**Fig. 4.** Observed concentration after administration of 10 mg cilobradine given as oral solution (**a**), oral capsule (**b**) and 20 min infusion (**c**), simulated median, 5th and 95th percentile concentration *vs* time.


a
Concentration [ng/mL]
Time [h]
Simulated concentration
5th and 95th percentiles
median
Observed concentration
subject # 301, 311, 312, 316 of study C
rest of study C
study A
b
Concentration [ng/mL]
Time [h]
Simulated concentration
5th and 95th percentiles
median
Observed concentration
subject # 311 of study C
rest of study C
study D
study E
c
Concentration [ng/mL]
Time [h]
Simulated concentration
5th and 95th percentiles
median
Observed concentration
subject # 301, 311 of study C
rest of study C
study B

(>$10^7$%). Without considering the parameter covariate relation the same estimates were obtained with even lower imprecisions and the same OFV (Table III, right panel). In addition, based on the final population PK model including the parameter covariate relation the majority of the observed high concentrations of the 2 lower dose groups (0.25 and 0.5 mg) were outside the 90% prediction interval (Fig. 5, small figures).

Both plots indicated that the external data set did not support the parameter covariate relation. Without the covariate relation all observations, even those of the low dose groups (Fig. 5, large figures) were well predicted with ≤13% of them being outside the 90% prediction interval. For all dose groups, the simulated median concentration-time profile reflected an overall slight underpredicting tendency of the model which was confirmed by the value of MDPE of −17%, MDAPE was 43%.

## DISCUSSION

In this study, a population PK analysis of the $I_f$ channel blocker cilobradine investigated in phase I clinical trials was performed. To date, PK analyses have been reported only for the two structural analogues zatebradine and ivabradine (30–33).

The developed population PK structural submodel of cilobradine was characterised by an open three-compartment disposition model, describing p.o. drug input by first-order kinetics and i.v. short infusion by zero-order kinetics. The elimination process was best described by first-order kinetics. The initial three-compartment model, although well describing all data (p.o. and i.v.), was intolerant to the exclusion of only few (2.2%) observations from six subjects. A three-compartment model based on the p.o. data only still described the p.o. data equally well but resulted in different estimates for KA and the ratios of the rate constants. The different estimates suggested a flip-flop situation. Depending on whether the p.o. data were modeled alone or together with i.v. data, the parameters were estimated very differently. Moreover, when modeling only p.o. data with all disposition parameters fixed to the final i.v. estimate, a relatively high KA value of 1.92 $h^{-1}$ was estimated. Fixing in the next step the KA value to this value and estimating all disposition parameters based on p.o. data only, all estimates were in the range of the final i.v. estimates reported in the manuscript. A reason for the differences that suggested a flip-flop situation might be that the sampling schedule during the first hour only contained a small number of p.o. data points. In order to obtain a robust model, a separate estimation of the distribution volumes V2, V3, and the inter-compartmental clearance Q3 for i.v. or p.o. data in a common model simultaneously fitting the p.o. and i.v. data was performed. In theory, there is an identifiability issue associated with the model. It has a priori two solutions resulting in model predictions that are impossible to distinguish and, theoretically, may have accounted for the need to use different V3, V2 and Q3 parameter values for i.v. and p.o. When simulating typical profiles, this complex model led to the same results as a simple model not distinguishing peripheral distribution volumes or inter-compartmental clearance between i.v. and p.o. As the more complex model was more robust without increasing the variance and also tolerant to the exclusion of the mentioned six subjects, still yielding similarly good results, it was selected for covariate analysis.

Absolute bioavailability F1 for p.o. solution or p.o. capsule was determined to be 34 or 43%, respectively. Surprisingly, absolute bioavailability after p.o. administration of the capsule was significantly higher estimated than for p.o. administration of the drinking solution [90% confidence interval of $F1_{sol}$ (29–

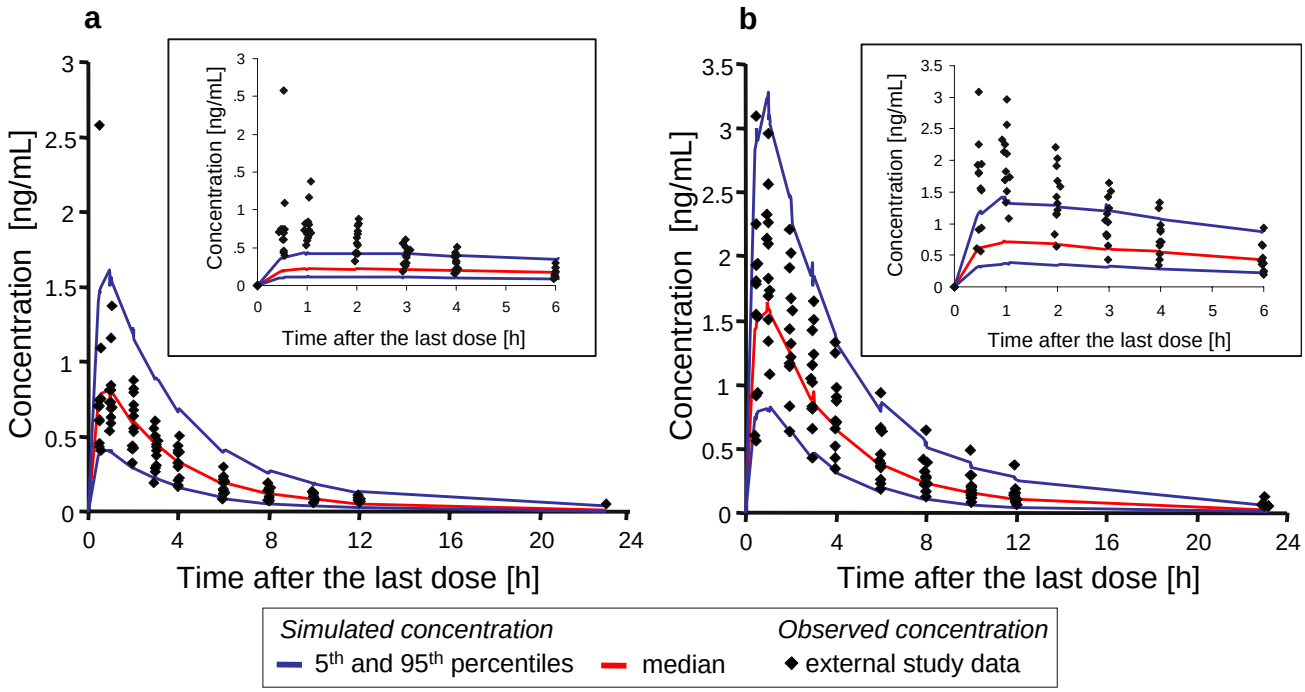



**Fig. 5.** Observed concentrations of the external data set after oral administration of 0.25 mg (**a**) and 0.5 mg (**b**) cilobradine given as solution, simulated median, 5th and 95th percentile concentrations *vs* time; simulations ($n = 500$) based on the final PK model not including the parameter covariate relation between KA and dose (*large figures*) and including the parameter covariate relation (*small figures*).

37%) and of $F1_{cps}$ (38–50%)]. The low values of F1 suggested first-pass metabolism and/or incomplete absorption. As the compound showed high solubility and high permeability first-pass metabolism is the more likely explanation (internal communication). For ivabradine and zatebradine, similar absolute bioavailability values have been reported and for zatebratine first-pass metabolism was shown (31,32). Thus, the existence of first-pass metabolism and its mechanism should to be further addressed for cilobradine.

Total clearance CL was estimated to be 21.5 l/h (=358 ml/min), indicating a moderate elimination capacity for cilobradine. As total CL exceeded the renal clearance (60–80 ml/min after i.v.; Boehringer Ingelheim, proprietary, confidential study report) the additional involvement of extrarenal elimination processes (e.g. metabolism) must be assumed. In accordance, metabolites of zatebradine have been detected in plasma, urine and faeces (31).

Typical steady state distribution volumes $V_{ss}$, calculated from estimated population values of V2, V3, V4 for p.o. and i.v., were large (95.8 and 130 l), suggesting an extensive distribution of cilobradine. Similar values of $V_{ss}$ have been found for zatebradine and ivabradine by individual compartmental and non-compartmental analysis, respectively (http://www.emea.eu.int/humandocs/PDFs/EPAR/procoralan/32044705en6.pdf) (31,32). In contrast, population PK analysis of ivabradine yielded an unusually high $V_{ss}$ of 860 l which the authors did not further comment (33).

The only parameter estimated with relatively high imprecision (52%) was Tlag which is not an uncommon finding and is probably due to the lack of data at early time points of 10 min and less after administration. The use of e.g. a transit-compartment model may improve the estimation of the time between drug administration and the first measured concentration as it better describes the mechanism of drug absorption (34). For our purpose, however, it was sufficient to describe the PK profile and variability of cilobradine with the model used in this study.

The terminal half-life ($t_{1/2,\,z}$) of cilobradine was calculated to be 29 h for p.o. and i.v. administration. Based on the published population PK estimates for ivabradine[33] the terminal half-life of ivabradine was determined to be in the same range. The reason why the value for zatebradine is much lower (2.4 h for p.o. and 2.8 h for i.v. administration) might be due to the much shorter sampling period of only until 9 h after administration compared to our schedule (31).

In the pharmacostatistical submodel, two hierarchical levels were identified for the variability by random effects: The inter-individual variability $\omega^2$ and the residual variability $\sigma^2$. Inter-individual variability estimated for CL, $F1_{sol/cps}$, central volume of distribution for i.v. ($V2_{iv}$) and absorption rate constant (KA) was moderate (15 to 46%), residual variability was low (26%). Regarding F1, formulation-dependent variability within one subject was better than only subject-dependent variability. Theoretically, an inter-occasion variability on F1 could have also explained the random differences occurring in the cross-over *study C*. However, the incorporation of an inter-occasion variability in the model was not successful probably due to the data available not being sufficient to allow for reliable determination of this parameter.

One further goal of the population approach was to assess the importance of the standard demographic and laboratory patient characteristics for an explanation and prediction of the inter-individual differences in the plasma concentration *versus* time profiles which has not been performed for any other $I_f$ channel blocker before. Only the parameter covariate relation between KA and dose was statistically significant. The relation was best described by a positive saturation function, where the parameter values indicated that the relation was primarily acting in the low dose range. The incorporation of the parameter covariate relation improved the model fit for few concentrations only. The exponential relationship was obviously forced by the two to three lower dose groups which consisted of a limited number of subjects (13–27% of total study population). When focusing on higher dose groups, no relationship was evident. Moreover, the covariate relation was not supported by external data. Additionally, implementation of the covariate relation led to a merely slight reduction of IIV of KA by only 12%. This suggested that our finding did not represent typical characteristics of cilobradine and is most probably of minor clinical relevance. The covariate relation between dose and KA has nevertheless been implemented into the final model, as it led to a statistically significantly better fit when using the development data set. The application of the model for data from subsequent studies will allow a final decision on the maintenance of the covariate relation in the final model and a possible mechanistic explanation. Overall, other demographic, laboratory or study-specific parameters did not show any statistically significant influence on the PK of cilobradine in healthy subjects. The relevance of all covariates in the target patient population should nevertheless be investigated.

Simulations of the development data set based on the final model showed sufficient predictability for the concentrations measured. The simulated median concentration-time profile reflected a very slight underpredicting tendency of the model which was confirmed by the value of the median prediction error of −4% with zero being between the 5th and 95th percentile. Beyond this internal evaluation the final model (without dose on KA) was successfully applied to predict external phase I data of cilobradine with a different population of volunteers and partially different dose groups. This result documented the robustness and general applicability of the developed model for the prediction of cilobradine concentrations of different origin.

Population PK models help to determine doses and dosing schedules for desired drug concentrations (35). Whereas the effective dose and the therapeutic index of cilobradine have not yet been determined, phase I studies have already shown acceptable tolerability of the drug. Therefore, the clinical relevance of the very slight underpredicting tendency of the model is probably negligible.

## CONCLUSION

In summary, in this study a reliable population PK model was developed for the new $I_f$ channel blocker cilobradine based on complex phase I data. Its general applicability for cilobradine was confirmed by external evaluation using phase I data. Population analysis additionally provided measures of imprecision (in total generally low) and of different types of variability which enables the better performance of clinical trial simulations. This model may form the basis for following

PK and pharmacodynamic investigations and for the development of rational dosing regimens for further clinical trials, e.g. with patients suffering from ischemia.

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