Pharmaceutical Research, Vol. 23, No. 8, August 2006 (© 2006)
DOI: 10.1007/s11095-006-9020-7

Research Paper

In Silico Modeling of Non-Linear Drug Absorption for the P-gp Substrate Talinolol and of Consequences for the Resulting Pharmacodynamic Effect

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Received August 24, 2005; accepted March 23, 2006

Purpose. The aim of the present work was to demonstrate P-glycoprotein's involvement in the non-linear talinolol pharmacokinetics using an advanced compartment and transit model (ACAT) and to compare the results predicted from the model to the finding of a phase I dose escalation study with oral talinolol doses increasing from 25 to 400 mg.

Materials and Methods. Besides minimum input parameters for the compound (p*K*a(s), solubility at one or more pH's, P_{eff}, doses, formulation, diffusivity), physiological and pharmacokinetic properties, transporter data are included in these predictions. The simulations assumed higher expression levels in lower gastrointestinal regions, in particular in the colon, which is in accordance with the results of intestinal rat perfusion studies and intestinal distribution data from rats, catfishes, micropigs and humans reported in the literature. Optimized values for P-glycoprotein (P-gp) K_m and V_{max} were used for the final simulation results and for a stochastic virtual trial with 12 patients.

Results. Talinolol, a P-gp substrate, exhibits non-linear dose AUC relationship after administration of 25, 50, 100 and 400 mg immediate-release tablets. This dose dependency is due to a decrease of efflux transport caused by saturation of P-gp by talinolol. It was found that oral bioavailability increases after administration of higher doses of talinolol. The predicted bioavailability of the p.o. 25, 50, 100 and 400 mg doses of talinolol was 64, 76, 85, 94%, respectively. Pharmacokinetic parameters (AUC, C_{max}) from *in silico* simulations are within acceptable range comparing with data, observed *in vivo*. However, the *in vitro* value of K_m for talinolol's interactions with P-gp could not be used in the simulation and still reproduce the observed non-linear dose dependence. For each of the four doses, GastroPlus® was used to model pharmacodynamic (PD) response and to optimize the values of CL_e, E_{max}, and EC_{50} with the effect compartment linked indirectly to the central compartment. For all simulations, EC_{50} was 114 nM and E_0 was 83 bpm.

Conclusion. Comparison between the results of the *in vivo* study and the *in silico* simulations determined the quality and reliability of the *in silico* predictions and demonstrate the simulation of dose dependent absorption. In contrast to previous simulation work for the non-linear dose dependence of interaction with intestinal transporters or enterocyte metabolism, optimized K_m and V_{max} values were required to reproduce the clinically observed non-linear dose dependence. The model developed may be useful in the prediction of absorption of other P-gp substrates including pharmacodynamic consequences.

KEY WORDS: absorption; GastroPlus®; intestinal efflux; pharmacokinetics; P-glycoprotein; simulation; talinolol.

INTRODUCTION

The term *in silico* refers to the estimation of certain parameters with the aid of computer-based software programs. Terstappen and Reggiani give an overview of the different areas in drug discovery, in which *in silico* approaches are used (1). Among other methods, computer-based programs are employed for the discovery of new drug targets, the analysis of gene-expression, the search for new lead substances and the prediction of physico-chemical properties of drugs such as solubility and lipophilicity (2–5). In addition, several approaches regarding the *in silico* modeling of the pharmacokinetic behavior of drugs in the human body have been reported (6–9). These approaches are based on the LADMER model, a model which describes the route of a drug through the human body in six steps: liberation (L), absorption (A), distribution (D), metabolism (M), excretion (E), and response (R) (10).

For perorally administrated drugs, processes that may influence bioavailability can be described as follows:

$$F = fa(1 - Eg)(1 - Eh)$$

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Where fa is the fraction of the dose absorbed across the apical cell membrane of the enterocyte, Eg is the fraction of drug extracted over the gut wall, and Eh is the fraction of drug metabolized by the liver (10).

The model is useful for *in silico* predictions of bioavailability, in particular C_{max}, t_{max}, AUC and other pharmacokinetic parameters after administration of immediate-release tablets. There are few examples of commercially available simulation software focusing on different aspects in drug development and pharmaceutical research: PhysioLab®, IDEA®, WinNonLin® WinNonMix®, and GastroPlus®. A more detailed description of six different software programs for *in silico* predictions of ADME and pharmacokinetics and an evaluation of these programs is reported by Boobis *et al.* (11).

Drug absorption from the gastrointestinal tract can be very complex. It is influenced by numerous physico-chemical, physiological and formulation-related factors. Due to this complexity, simple models often fail to characterize the absorption process correctly. Models suggested for the simulation of the gastrointestinal tract have been the dispersion model (12), homogenous (13) and heterogenous (14,15) tube model and compartmental absorption and transit (CAT) model (16,17). Furthermore, for simulations of the absorption of drugs undergoing active transport, including both, uptake and efflux processes, a model may not focus on passive diffusion alone, but must provide simulation steps for the transporters involved. The advanced compartmental absorption and transit (ACAT) model implemented in the GastroPlus® software is able to simulate such active transport processes (9). In addition, the ACAT model accounts for factors such as variations in pH along the gastrointestinal tract, physico-chemical parameters of the drug molecule that affect dissolution and absorption, variations in effective permeability and structure in the intestine, physical formulation properties, and saturable first-pass extraction and biotransformation.

The objective of the present study was to demonstrate a prediction of non-linear dose-dependent absorption properties of talinolol using an *in silico* approach. Talinolol, (−/+)-1-(4-cyclohexyl-ureido-phenoxy)-2-hydroxy-3-tert.-butyl-aminopropan is a selective β-1 adrenoceptor antagonist without partial agonistic activity and membrane stabilizing activity, used in the treatment of hypertension, tachycardia dysrhythmias and coronary heart disease (18,19). Due to the β-1 adrenergic blockade it induces, talinolol has no detectable negative effects on glucose metabolism or lipid profile (19). β-1 and β-2 adrenoradioreceptor assay (RRA) confirmed that talinolol binds to β-receptors with moderate affinity but talinolol is a highly selective and efficient β-1 adrenoceptor antagonist. The S (-) enantiomer had an affinity about twice that of the racemate, but almost the same β-1 selectivity (20). Talinolol has a plasma half-life (11.9+/−2.4 h) which ensures the therapeutic effectiveness of a once-daily 100 mg dosage over the whole 24 h (18).

Talinolol was chosen as a model compound with regard to intestinal secretion, which was detected in *ex vivo* studies via transport inhibition with verapamil, in *in situ* intestinal perfusions in rats following a talinolol i.v. bolus, and *in vivo* studies in humans (21,22, and 23). Its biotransformation in man is negligible (<1% after an intravenous dose in humans). This ensures that the intestinal drug efflux can be observed without an interference with biotransformation processes. One metabolic pathway is the formation of 4-*trans* hydroxytalinolol, followed by the formation of 3-*cis*, 4-*cis* and 3-*trans* hydroxytalinolol (18,22,23, and 24). Talinolol shows minor additional P-gp dependent excretion routes. The results obtained from previous studies show that talinolol undergoes an enterohepatic circulation, but biliary secretion of the drug was less than 10% of an intravenous dose. It is assumed, that talinolol dose-dependence absorption cannot be fully explained by saturation of biliary excretion and that intestinal secretion is involved (23,25).

MATERIALS AND METHODS

Computer Hardware and Software

The simulations were performed on a Siemens Xpert Pentium III computer (500 MHz) using the GastroPlus® software (Simulations Plus, Lancaster, California, USA). The program enables predictions of rate and extent of drug absorption from the gastrointestinal tract. It also allows considering intestinal drug efflux and metabolism in its predictions of pharmacokinetic parameters.

Only a few basic parameters: p*K*a(s), solubility at one or more pH's, effective permeability, dose, formulation and diffusivity are initially required to run a simulation with GastroPlus®. However, there are a large number of additional input parameters that are not essential for a simulation but can enhance the quality of the results. For optimum predictability of pharmacokinetic profiles, detailed information on the properties of the particular drug and its dosage form is required.

Input Parameters for Simulations of Pharmacokinetics of Talinolol from IR Tablets

Compound Properties

The parameters available for talinolol are depicted in Table I. When no information was available concerning a certain input parameter, default settings proposed by the program were used.

Gastrointestinal Absorption Model

Absorption may occur throughout the gastrointestinal tract (GIT). Its particular segments are characterized by the relative ratio of transit and absorption rates. Passive absorption is a diffusion-controlled process, and the permeability of drug varies as a function of surface area to volume ratio and regional pH effects on drug ionization. According to Fick's law, the driving force for diffusion across the apical and basolateral membranes of the enterocyte is the soluble drug concentration gradient. For ionizable drugs this varies with the p*K*a and the pH profile between the intestinal compartments. The employed version of the ACAT model accounts for dissolution rate, pH dependence of drug solubility, release rate, absorption in the stomach, small intestine or colon, metabolism in gut or liver, degradation in the lumen, or changes in factors such as surface area, transporter

Table I. Basic Compound Parameters for Talinolol used in the Simulations

Drug properties:	
Molecular formula	$C_{20}H_{33}N_3O_3$
Molecular weight (M_r)	363.5 g/mol
Base p*K*a	9.43[a]
Reference logD	3.15 at pH 9.4
Permeability ($\times 10^{-4}$ cm/s)	1.68[b]
Solubility at pH 7.0	4.5 mg/ml[c]
Solubility at pH 7.4	1.234 mg/ml[a]
Solubility at pH 9.8	0.057 mg/ml[d]
Solubility factor	112[e]
Dosage form properties:	
Dosage forms	IR tablet i.v. bolus
Initial dose	p.o.: 25, 50, 100, 400 mg i.v.: 30 mg
Dose volume	200 ml
Mean precipitation time	900 s
Drug particle density	1.2 g/ml
Effective particle radius	25 μm
Diffusion coefficient	0.653×10^{-5} cm^2/s

[a] Literature value was taken from (22).
[b] Effective permeability was estimated using ADMET Predictor (Simulations Plus, Inc.).
[c] Literature value for solubility was taken from (25).
[d] Native solubility value was estimated using QMPRPlus (ver. 4.0.6)®-MeylanMLP.
[e] Solubility factor (fold difference between intrinsic and salt forms) was calculated from three solubility values.

densities (e.g., efflux protein densities), and other regional factors within the intestinal tract (9,26).

The ACAT model (Fig. 1) is a flexible physiologically based simulation of a single drug passing through the small intestine (SI) and the colon. Each compartment of the SI and the colon is modeled with accurate physiological information regarding volumes, transit times, length, and radius. Table II lists the physiological parameters that define the volumes, geometries, pH values, and transit times for the model used in this study.

The form of the ACAT model implemented in Gastro-Plus® is modeled by a system of coupled linear and non-linear rate equations. The equations include the consideration of six states (unreleased, un-dissolved, dissolved, degraded, metabolized, and absorbed), 18 compartments (the stomach, the small intestine, which is divided into seven compartments, the colon and nine enterocyte compartments), three states of excreted material (unreleased, undissolved, and dissolved), and the amount of drug in physiologically based organ compartments, when tissue partition and flow rate parameters are available (Table II). The total amount of absorbed material is summed over the integrated amounts being absorbed/exsorbed from each absorption/transit compartment.

The rate of change of dissolved drug concentration in a luminal GI compartment generally depends on six different processes: (1) transit of drug into a compartment, (2) transit of drug out of a compartment, (3) release of drug from the formulation in the compartment, (4) dissolution of the drug particles, (5) luminal degradation of the drug (if any), and (6) absorption/exsorption of the drug. The time scale associated with luminal transit is set by a transfer rate constant k_t, which is determined from the mean transit time within each compartment. The time scale of the dissolution process is set by a rate constant, k_d, that can be computed from a drug's solubility (as a function of pH), its effective particle size, its molecular density, its lumen concentration, its diffusion coefficient, and the diffusion layer thickness. The time scale associated with the absorption process is set by a rate coefficient k_{ai}, which depends on the product of effective permeability of the drug multiplied by an absorption scale factor (ASF, with units of cm^{-1}, a function of pH and log D).

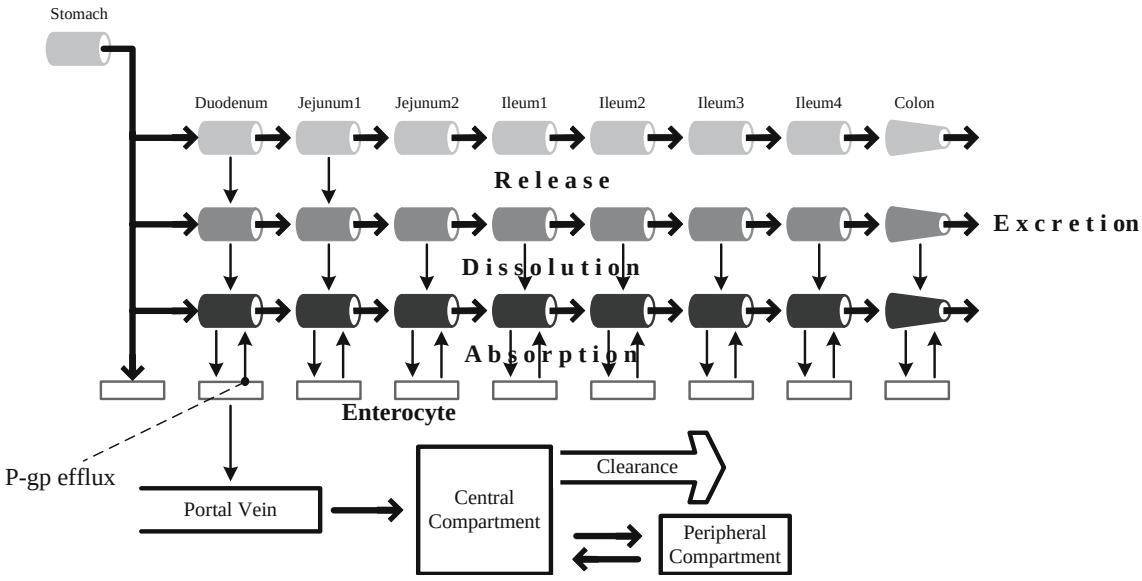

Fig. 1. Schematic representation of the process of talinolol absorption following oral administration: transit model segmenting GIT into different compartments (stomach, seven small intestine compartments, colon and nine enterocytes). Administered drug, after dissolution, becomes available for passive absorption and efflux secretion. The rate of drug transfer into and out of enterocyte compartment for each GIT lumen compartment is calculated using the concentration gradient across the apical and basolateral membranes (9).

The ASF corrects for changes in permeability due to changing physiology along the GI tract; e.g., surface area available for absorption, pH and log D of the drug molecule. The rates of absorption and exsorption depend on the concentration gradients across the apical and basolateral enterocyte membranes. P-glycoprotein (P-gp), an ATP-driven efflux pump, is generally considered to have a physiological distribution that increases aborally. Several studies in rats and humans have helped to confirm this general trend for the distribution of P-gp (27). The data in animals and humans all show the same trend in the regional distribution of P-glycoprotein in the small and large intestine. Regional distribution of P-gp in rats (28,29) and catfish (30) are similar, suggesting an increase of P-gp levels in the ileum and colon. In micropigs (31) P-gp mRNA expression is reported to be higher in proximal parts of the small intestine but Western blot of the Pgp protein expression also shows higher levels in the distal portions. As a result of the *in vivo* studies in different regions of the human intestine (32) the scaling factors for P-gp distribution were adopted.

The distribution of P-glycoprotein listed in Table II and used for this study was taken from the experimental distribution in human tissue (32).

The system of differential equations is integrated using the Livermore Solver of Ordinary Differential equations with Automatic method switching for stiff and non-stiff problems (33). The fraction of dose absorbed is calculated as the sum of all drug amounts disappearing from the GI tract as a function of time, divided by the dose, or by the sum of all doses if multiple dosing is used. Bioavailability is calculated as the fraction of dose reaching the systemic circulation, and is distinguished from absorption, which is the fraction of dose crossing the apical membrane of the enterocyte.

Disposition Parameters for Talinolol

Pharmacokinetic (PK) parameters were determined by using the "PKPlus" module. This program will accept i.v. bolus or infusion data and will determine the best fit to one-, two, and three-compartmental PK models.

The K_m (Michaelis-Menten constant) and V_{max} (maximum transport rate for efflux transporter) for talinolol, used in this study, were optimized by GastroPlus®. Intravenous bolus (30 mg) and oral doses (25, 50, 100, and 400 mg), which have been administered to human volunteers and the plasma concentration *vs*. time resulting data were taken from the doctoral thesis of Wetterich (34). Due to the fact that the biotransformation of talinolol accounts for less than 1% (24,35), the parameter for the first-pass extraction was set to 0%. The unbound fraction in plasma was calculated as the difference between 100% and the fraction bound to plasma proteins (i.e., 55% (36)):

$$F_{unbound} = 100\% - 55\% = 45\%$$

Both unbound and total serum talinolol concentrations were measured using ultrafiltration and a HPLC method. Ultrafiltrates were obtained by centrifugation at 2,000 rpm for 20 min at room temperature. No measurable adsorption of talinolol onto the ultrafiltration membrane was noted (36).

Virtual Trial (Stochastic Simulation)

The Virtual Trials feature in GastroPlus allows the user to assess the combined effects of variations in population physiology and formulation variables that are not precise values, but for which distributions of values can be estimated. The coefficient of variation (CV%) values were determined the basis of our previous experience and analysis of the literature. Such parameters include physiological variables such as transit times in the various compartments, pH's in all compartments, pharmacokinetic parameters, plasma protein binding and renal clearance. Stochastic simulations based on the means and the log normal distributions were used with the following coefficients of variation:

Gastrointestinal pH, lengths and radii = 10%
Transit times and pharmacokinetic distribution parameters = 20%
Hepatic perfusion = 30%
Systemic clearance = 40%
Pgp V_{max} and K_m = 50%
Passive permeability = 65%

Random samples of all of the stochastic variables were generated for each simulation. The final results were based on a virtual trial with 12 patients which is equal to the number of patients used for the experimental clinical data.

RESULTS AND DISCUSSION

Selection of an Adequate Disposition Model

The compartment model for all simulations was selected on the basis of human plasma data after an intravenous bolus

Table II. ACAT Physiological Model Parameters used in Current Simulations

Compartment	ASF (cm^{-1})	pH	Transit time (h)	Volume (ml)	Length (cm)	Radius (cm)	P-gp Expression (relative)[a]
Stomach	0	1.5	0.75	50	30	N/A	0
Duodenum	1.46	5.0	0.25	113	25	1.2	0.538
Jejunum 1	1.82	6.0	0.51	157	50	1.0	0.645
Jejunum 2	1.86	6.3	0.51	157	50	1.0	0.723
Ileum 1	3.76	6.6	0.68	54	67	0.51	0.770
Ileum 2	3.90	6.9	0.68	54	67	0.51	0.838
Ileum 3	4.04	7.2	0.68	54	67	0.51	0.908
Caecum	0.32	5.0	4.5	269	7	3.5	1
Ascending colon	1.02	7.5	13.5	570	29	2.5	1

[a] Relative P-gp levels were expressed as the ratio of the IOD (integrated optical densities) of P-gp to that of villin (32).

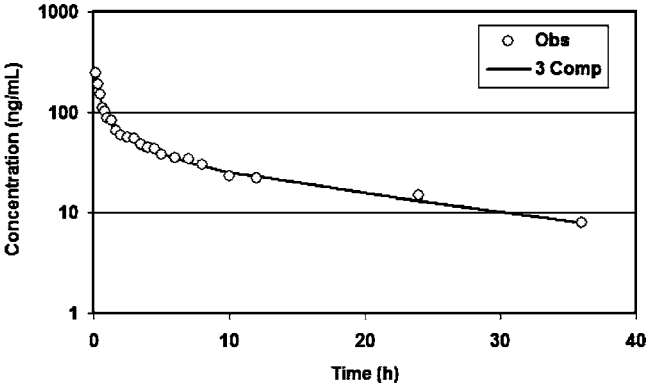

Fig. 2. Fit of three-compartment model to plasma data from a human study with 30 mg talinolol administered as i.v. bolus.

of 30 mg talinolol. The data were fit to a one-, two- and three-compartment pharmacokinetic model.

Figure 2 illustrates that the three-compartment model provides the best fit of the data. For the one-compartment model deviations were rather distinct, whereas the two-compartment model over predicts at 10 h and under predicts at 35 h. Two different initial distribution phases can be observed, arguing for the three-compartment model. A three compartment pharmacokinetic model was selected on the basis of the lowest value of the Akaike information criterion (23, −22, and −56 for the 1, 2, and 3 compartment models, respectively) (37), that is in accordance with results reported by Wetterich *et al*. (23).

The value for the model derived clearance lies within the range reported by Wetterich *et al*. (0.382 *vs*. 0.253–0.811 l/h/kg), whereas the value suggested for the volume of the central compartment is higher than reported (1.211 *vs*. 0.2–0.5 l/kg) (23).

Optimization of the transporter parameters K_m and V_{max}

Michaelis-Menten parameters K_m and V_{max} determine the extent of the intestinal drug efflux as well as the saturation level of the efflux pump. For the interpretation of the parameter optimization results, it is important to understand the meaning of the parameters K_m and V_{max}. In the case of transport-mediated reactions the two parameters K_m and V_{max} characterize the properties of transport proteins. High V_{max} values refer to transporters with high turnover rates and low K_m values indicate that a transporter reaches its saturation level at low drug concentrations. Since actual values are not directly measurable, V_{max} and K_m can only be estimated for the mediated secretion in the human intestine.

In order to obtain good correlations between *in silico* simulations and *in vivo* data, optimization of the *in vitro* P-gp binding parameters using GastroPlus® was performed. Intracellular enterocyte concentrations are a function of the rate of transfer across the apical and basolateral membranes. The rate entry into and out of the enterocyte compartment depends on V_{max} and the passive transmembrane permeability. As part of the GastroPlus® validation process, the non-linear dose dependence of gut wall first pass metabolism for midazolam was accurately simulated using the experimental *in vitro* K_m values and the intra-enterocyte concentrations generated by the default ACAT model (9). Simulation of plasma concentrations following a single dose of talinolol could be accomplished with many combinations of K_m and V_{max}. On the assumption that the ACAT compartment model is correct and that the estimate of passive transcellular permeability from QMPRPlus® is a good estimate of the passive component of human effective permeability, then a unique solution to the optimization of K_m and V_{max} for P-gp efflux of talinolol can be found. Accordingly, a single value for K_m and V_{max} was optimized for all four doses simultaneously.

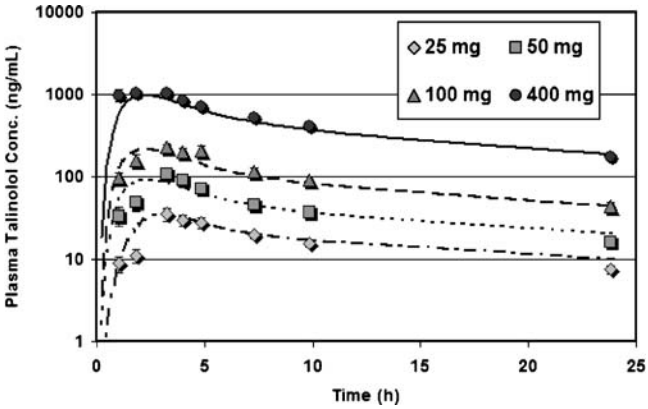

Fig. 3. Plasma concentration-*versus*-time profile after administration of immediate-release tablets simulated by GastroPlus®. The symbols indicate mean plasma concentrations observed *in vivo* after administration of the IR tablets with standard error bars. Disposition parameters for talinolol used in current simulations for all doses: K_{12}: 1.393, K_{21}: 0.948, K_{13}: 0.492, K_{31}: 0,136 (the micro-constants for transfer of drug to the second and third compartments, h^{-1}), CL: 0.382 (clearance, $L^* h^{-1*} kg^{-1}$), V_c: 1.211 (volume of the central compartment, $L^* kg^{-1}$), F_{pu}: 45 (fraction unbound in plasma, %).

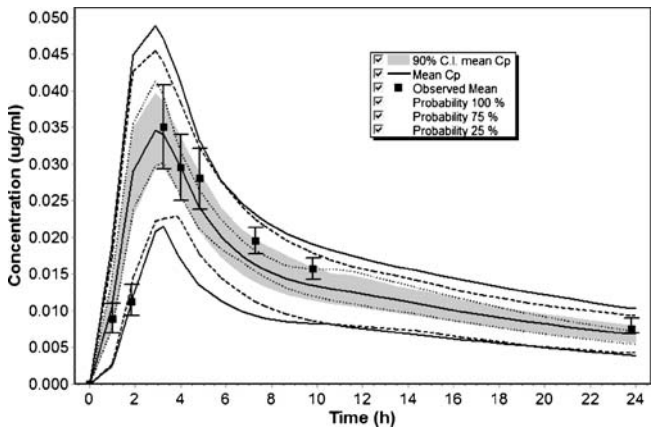

Fig. 4. Stochastic virtual trial simulation for 12 patients at an oral dose of 25 mg. A *solid black line* near the middle of the Cp *vs*. time profile represents the mean of 12 simulations. *Solid black squares* with standard error bars represent the observed clinical Cp *vs*. time data. The *grey shaded area* represents the 90% confidence interval for the simulated data, and the *solid, dashed, and dotted lines* represent individual simulated results that include 100, 75, and 25% of the range of simulated patient data.

Table III. Simulation Results for Immediate-Release Formulations Containing 25, 50, 100 and 400 mg Talinolol

Dosage form	Bioavailability predicted (%)	Cmax (ng/ml) (*In vivo* study)	Cmax (ng/ml) (predicted)	AUC (0–24 h) (ngh/ml) (predicted)	AUC (0–24 h)/Dose (predicted)	AUC (0–24 h)/ Dose *in vivo* study
IR 25 mg	64	39.6 ± 17.3	34.6	385	15.4	14.4 ± 4.87
IR 50 mg	76.2	124.7 ± 49.2	90.8	942	18.8	18.4 ± 6.33
IR 100 mg	84.8	234.3 ± 108.5	215.0	2125	21.2	22.6 ± 6.48
IR 400 mg	93.7	1270.5 ± 436.9	985.0	9479	23.7	27.1 ± 5.23

The increases in bioavailability and the AUC-dose-ratio demonstrate a dose dependence caused by the saturability of the intestinal drug efflux. For comparison AUC-dose-ratios (Means ± S.D.) observed *in vivo* are added (34).

The optimization resulted in a value of 0.25 μg/ml (0.69 μM) for K_m and 0.00178 mg/s for V_{max}. These results are not consistent with measured *in vitro* values. Radioligand binding studies indicate that the IC_{50} for talinolol interaction with P-gp has two sites with affinities of 72 and 1,570 μM. The estimated composite K_m for P-gp is 412 μM. Thus, the optimized value of 0.69 μM is too low to match the *in vitro* values. One possible explanation would be that the underlying enterocyte section of the ACAT model in GastroPlus® is not correct. The volumes of the enterocyte compartments could be too large resulting in lower intracellular concentrations which would require that the simulated K_m would need to lower to simulate the observed non-linear dose dependence. However, the validation of the default ACAT model using multiple doses of midazolam would suggest that the concentrations in human enterocytes are correct.

We hypothesize that the reason for the difference between the *in vitro* value for K_m and the optimized *in silico* value is due to the fact that the ACAT model is using the simulated concentration of the cytoplasm of the enterocyte for interaction with the binding site of P-gp. The ACAT model predicts a very low concentration because of the rapid influx and efflux of talinolol in the enterocyte. Whereas, the concentration of talinolol within the apical bilayer membrane would be expected to be much higher due to the hydrophobicity of the molecule allowing for rapid entry into the bilayer and relatively slower exit from the bilayer (38). It is also well known that the binding sites for P-gp lie within the apical leaflet and those substrates are thought to enter the membrane and diffuse laterally for interaction with P-gp. This limitation of the ACAT model might be avoided by calculating the much higher intra-membrane concentration for hydrophobic molecules like talinolol.

Simulations with Orally Administered Immediate-Release Tablets Following Talinolol Single Doses of 25, 50, 100 and 400 mg

Simulation of bioavailability and plasma concentrations—*versus* time curves for immediate-release tablets and doses of 25, 50, 100 and 400 mg of talinolol were performed using the i.v. pharmacokinetics and experimental or estimated values for the biopharmaceutical properties as in Table I. All doses were simulated using the same values for the transporter parameters K_m and V_{max} as discussed above.

The route of the administered dosage form through the different regions of the gastrointestinal tract with respect to the sites of dissolution and absorption can be observed during a simulation. The simulation results along with the mean plasma concentration *vs*. time data and standard error bars are presented in Fig. 3 for immediate-release tablets and 25, 50, 100, and 400 mg doses of talinolol.

The PK study in humans demonstrated non-linear dose dependence due to saturation of P-gp. It was found that by increasing the oral dose, the AUC increased more than proportional with dose (23). This phenomenon was accurately simulated *in silico* by the model developed using the GastroPlus® software.

Simulations for immediate-release dosage forms and single doses of 25, 50, 100, and 400 mg of talinolol were compared with the plasma concentration—*versus* time curves observed *in vivo*. The results of a virtual trial for 12 patients at an oral dose of 25 mg are shown in Fig. 4. This figure shows the mean Cp *vs*. time profile, the experimentally observed values with their standard error bars, and has a grey colored area that represents the 90% confidence intervals around the mean. In addition, the solid lines labeled as 100% probability represents the highest and lowest Cp *vs*. time curves from the 12 patients in the virtual trial. The dotted and dashed lines represent corresponding Cp *vs*. time curves for 25 and 75% probability, respectively. It can be seen that all but one of the experimental clinical observations lies within the 90% confidence intervals of the simulation. Also, it can be seen that the all of the observed data lies within the minimal and maximal individual patient simulations from this 12 patient virtual trial. This would suggest that the CV % values used for the log normal distributions produced simulated variability that encompasses the observed clinical results.

Figures 3 and 4 show both the variability associated with the observed clinical data as well as the variability that would be expected from the simulations given our best assumptions

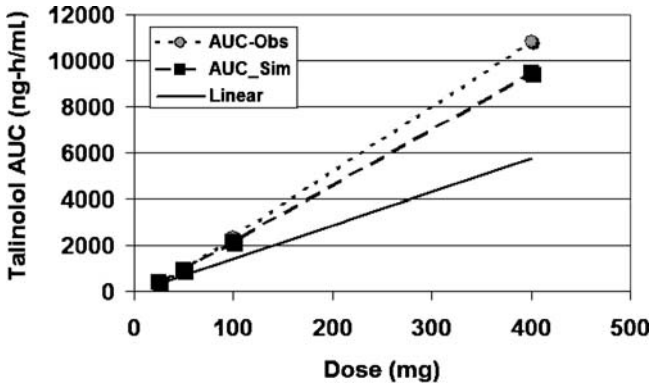

Fig. 5. Differences between linear and non-linear dose-AUC relationship after oral administration 25 to 400 mg talinolol doses.

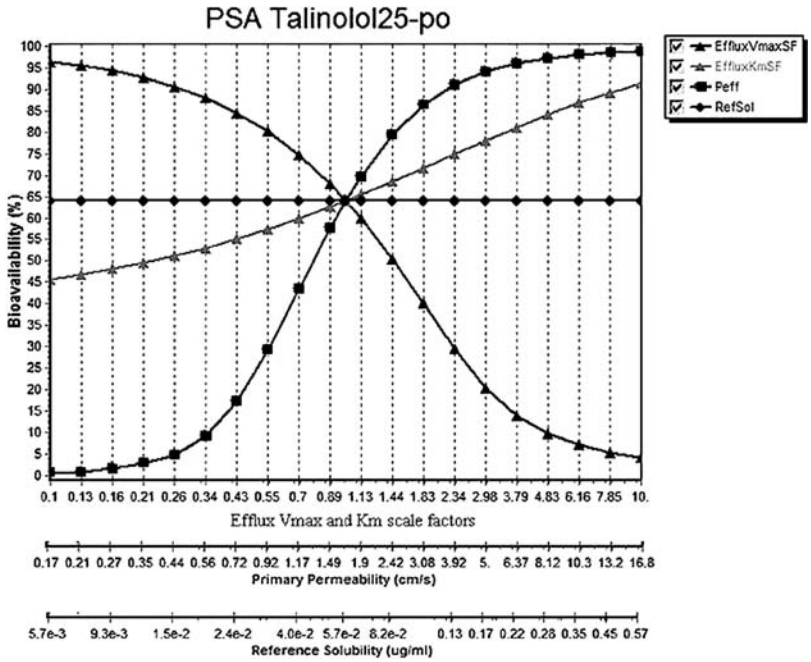

Fig. 6. Parameter sensitivity analysis for 25 mg talinolol p.o. administration. The *Y*-axis is the simulated bioavailability. The center of the *X*-axis for each of the parameters tested represents the value that was used in the simulations shown in Fig. 3. Each of the *X*-axis scales show the direction and magnitude of the perturbation from the initial parameter value.

for the coefficients of variations for individual physiological, biochemical, and pharmacokinetics parameters.

The simulation results demonstrate acceptable predictions of the *in vivo* plasma profiles. Most of the predicted plasma concentrations lie in the range between the minimum and maximum plasma levels obtained in the *in vivo* study. Furthermore, the predicted parameters C_{max} and AUC are located within one standard deviation of the mean experimental parameters in 12 volunteers (Table III).

After *in silico* prediction in which 400 mg IR talinolol was used as dose and formulation inputs, plasma concentrations were lower than was expected according to the *in vivo* results (34). For the 400 mg dose, the calculated Dose number was 2.2 indicating that there might be a problem in solubilizing that much talinolol. The observed results provide additional validation of the ability of the software to simulate limited solubility drugs correctly. Both the simulated and *in vivo* results suggest that the GI fluids are capable of dissolving talinolol and precipitation is avoided during the movement of compound through the gut due to the high pKa value of 9.43, even after administration of IR tablets at doses as high as 400 mg of talinolol.

The results given in Table III present that bioavailability increases with higher doses (increase in AUC-dose ratio). These findings are important to demonstrate that the model is capable of simulating effects of non-linear dose dependence caused by intestinal drug efflux.

Table III also shows the deviations between the AUC-dose ratios calculated from the *in silico* simulations and from the *in vivo* data, which are very low, particularly considering that the absorption and elimination process *in vivo* is so complex. Such an accurate simulation result would be even more impressive if the *in vitro* values of P-gp parameters (K_m and V_{max}) for talinolol were available and could be used

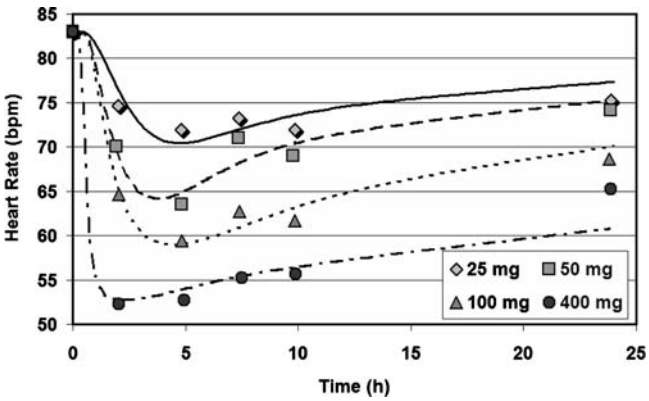

Fig. 7. Pharmacodynamic optimization of the change in heart rate during a four-minute supine bicycle exercise period.

Table IV. Pharmacodynamic Parameters of Indirect Link: Effect Compartment Model

Dose	E_{max} (bpm)[b]	E_{max} (Rel. %)[c]	CL_e (h^{-1})
25	−57.7	50.7	0.466
50	−40.9	84.4	0.802
100	−38.0	90.2	0.490
400	−33.1	100	8.12

[b] E_{max} value as used in equation (2); For all simulations, EC_{50} was 114nM and E_o was 83bpm

[c] E_{max} as a relative % change in bpm ((83-E_{max})/49.9bpm); (49.9bpm is derived from 83 + E_{max} for 400mg)

without optimization. Such a result would not be possible without an ACAT model that correctly represents the *in vivo* human gut distribution of P-gp and simulates the lumen and enterocyte concentrations accurately. It must be stressed that all of the biopharmaceutical properties were derived from *in silico* estimates from structure or *in vitro* data. Regional absorption scale factors and formulation factors such as particle size, density, and drug diffusivity were default values and were held constant for all simulations.

At doses of 25 and 50 mg of talinolol the concentrations of the substrate was below saturation levels. Increase in the dose results in a more than proportionate increase in the AUC. The source of this nonlinearity may be explained in saturation of the intestinal efflux pump.

Figure 5 represents dose-AUC relationship which, in the case of linear pharmacokinetics is a straight line and when efflux mechanisms are involved in the absorption has an increasing slope.

Preliminary simulations demonstrated that the transporter parameters K_m and V_{max} (Michaelis-Menten parameters) have a pronounced influence on the *in silico* results. GastroPlus® was used to conduct a parameter sensitivity analysis for the Michaelis-Menten constants, passive transcellular permeability, and solubility. The results are shown in Fig. 5.

Figure 6 shows that bioavailability is very sensitive to changes in V_{max} and passive transcellular permeability with less sensitivity to changes in K_m and zero effect from changing solubility by an order of magnitude increase or decrease. This result emphasizes the fact that optimization of the appropriate K_m and V_{max} that will reproduce the non-linear dose dependence is very complex. In addition, high inter-and intraintestinal variations in expression of P-gp among healthy volunteers (32), would be reflected in large changes in bioavailability due to small changes in V_{max} and passive transcellular permeability.

Pharmacodynamics

de Mey *et al*. (20) reported the change in heart rate during five sessions of a four-minute supine bicycle exercise period after p.o. administration of talinolol. The first session was conducted at 2 h after p.o. administration of talinolol at 25, 50, 100, and 400 mg. Four other exercise sessions were conducted at 5, 7.5, 10, and 24 h. GastroPlus® was used to model this pharmacodynamic (PD) response in order to determine the *in vivo* EC_{50} and maximal response values for an Indirect Link: Effect Compartment PD model. In this PD model drug concentration in effect compartment, which is connected to the central compartment, determines the PD effect.

The effect compartment model proposes that the delay in the pharmacologic action of the drug from the time of entering the plasma compartment is due to redistribution to the effect compartment, the true site of the pharmacologic action. If A_e is the amount in the effect compartment, then the rate of transfer between the central compartment and the effect compartment can be written as clearance Eq. (1):

$$\frac{dA_e}{dt} = CL_e(C_p - C_e) \tag{1}$$

Where:

CL_e is the clearance rate between central and effect compartments in liters per hour

C_p is the plasma concentration

C_e is the concentration in the effect compartment

Once the drug has entered the effect compartment the effect on heart rate can be calculated from a normal sigmoidal dose response relationship.

$$E = E_0 + \frac{E_{max}C}{EC_{50} + C} \tag{2}$$

For each of the four doses, GastroPlus®-PDPlus was used to optimize the values of CL_e, E_{max}, and EC_{50} with the effect compartment linked indirectly to the central compartment. E_0 was held constant at the average resting heart rate of 83 beats per minute (bpm). In the first round of optimization, each dose was optimized individually and the individual EC_{50} values were averaged to give a mean (S.D.) of 114 nM (±49 nM). In a second round of optimization we held the EC_{50} value constant at 114 nM for each dose and optimized only the E_{max} and CL_e values. Table IV lists the final optimized pharmacodynamic parameters used in Fig. 7.

In conclusion, a combined pharmacokinetic/pharmacodynamic model has been developed for talinolol which allows predictions of pharmacokinetics and effect for a compound undergoing nonlinear drug absorption due to P-gp mediated intestinal secretion. Previous validation studies have shown that *in vitro* parameters for influx gastrointestinal transporters like PepT1, HPT1, and amino acid transporters as well as enterocyte metabolism could be used to reproduce the non-linear dose dependence observed *in vivo*. This study highlights a deficiency in the ACAT model for prediction of the non-linear dose dependence of substrates for efflux transporters when only *in vitro* data is available. Further studies should aim at validating this model using velocities of drug input different from immediate release formulations such as slow release or pulsatile releasing dosage forms.

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