Pharm Res (2012) 29:574–584
DOI 10.1007/s11095-011-0595-2

RESEARCH PAPER

A Semi-mechanistic Modeling Strategy for Characterization of Regional Absorption Properties and Prospective Prediction of Plasma Concentrations Following Administration of New Modified Release Formulations

Martin Bergstrand • Erik Söderlind • Ulf G Eriksson • Werner Weitschies • Mats O Karlsson

Received: 30 March 2011 / Accepted: 14 September 2011 / Published online: 23 September 2011

ABSTRACT

Purpose To outline and test a new modeling approach for prospective predictions of absorption from newly developed modified release formulations based on *in vivo* studies of gastro intestinal (GI) transit, drug release and regional absorption for the investigational drug AZD0837.

Methods This work was a natural extension to the companion article *"A semi-mechanistic model to link in vitro and in vivo drug release for modified release formulations"*. The drug release model governed the amount of substance released in distinct GI regions over time. GI distribution of released drug substance, region specific rate and extent of absorption and the influence of food intake were estimated. The model was informed by magnetic marker monitoring data and data from an intubation study with local administration in colon.

Results Distinctly different absorption properties were characterized for different GI regions. Bioavailability over the gut-wall was estimated to be high in duodenum (70%) compared to the small intestine (25%). Colon was primarily characterized by a very slow rate of absorption.

Conclusions The established model was largely successful in predicting plasma concentration following administration of three newly developed formulations for which no clinical data had been applied during model building.

KEY WORDS IVIVC · magnetic marker monitoring · mechanistic modeling · modified release · NONMEM

INTRODUCTION

Sophisticated *in vivo* methods to study gastro intestinal transit and/or regional absorption of pharmaceuticals are increasingly used in drug development. This work aims at developing suitable model based approaches to analyse data from studies with some of these techniques and to demonstrate the value that they can add in terms of prediction of plasma concentration profiles for new modified release (MR) formulations.

Gamma scintigraphy was introduced as the first fairly convenient means of studying gastro intestinal transit and *in vivo* disintegration of solid dosage forms (1–3). Magnetic Marker Monitoring (MMM) has lately become an attractive alternative that avoids the radiation associated with the gamma scintigraphy method (4). The MMM technique is based on the determination of the magnetic dipole moment generated by magnetically labeled solid dosage forms. With the MMM technique the disintegration properties of the solid dosage form can be monitored during its passage through the GI tract by means of the decrease of magnetic

M. Bergstrand (✉) · M. O. Karlsson
Department of Pharmaceutical Biosciences, Uppsala University
P.O. Box 591, SE-751 24 Uppsala, Sweden
e-mail: martin.bergstrand@farmbio.uu.se

E. Söderlind
Pharmaceutical Development, AstraZeneca R&D
Mölndal, Sweden

U. G. Eriksson
AstraZeneca R&D
Mölndal, Sweden

W. Weitschies
Institute of Pharmacy, University of Greifswald
Greifswald, Germany

moment. For dosage forms where the drug release rate is determined by the erosion of the dosage form, the decrease in magnetic moment can be linked to the drug release. In these cases a relationship between decrease in magnetic signal and drug release characterized in *in vitro* experiments can be used to obtain actual *in vivo* drug release profiles (5). The authors have previously demonstrated the possibility to characterize regional absorption properties with application of population pharmacokinetic principles to data from an MMM study (6). Regional differences in rate and extent of absorption in different segments of the GI tract have otherwise more commonly been studied with gastro intestinal intubation methods (7). An example of such an intubation method is the Bioperm® capsule technique (8). The method features a thin tube introduced through the nose, retrieved from the pharynx, attached to a 30 mm long capsule, and swallowed. Peristalsis moves the capsule to the desired location in the gut (monitored by X-ray) where it is anchored before administration via the tube.

AZD0837 is a novel oral direct thrombin inhibitor developed by AstraZeneca and investigated in clinical phase II studies for the prevention of stroke in arterial fibrillation patients (9,10). It is a prodrug that is bioconverted via an intermediate to its active form, AR-H067637. Development of a suitable extended release (ER) formulation was undertaken to support a once daily dosing (11). Two mechanistic absorption studies have been performed with AZD0837 to guide the development of a suitable ER formulation. In one study Bioperm® capsule was used to study the absorption in colon. Another study with the MMM technique was performed to study *in vivo* drug release, gastro intestinal transit and regional absorption for a magnetically labeled ER formulation.

The work presented in this manuscript builds on a simultaneously submitted article "A mechanistic model to link *in vitro* and *in vivo* drug release for modified release formulations" (12). In that article a model describing *in vitro* drug release for a group of AZD0837 candidate formulations was described. The *in vitro* model was linked to the *in vivo* conditions based on observations of *in vivo* drug release in the MMM study (Table I, Study 1). Furthermore that article includes the description of a model for tablet GI transit with or without concomitant food intake. The drug release model established in the previous article acts as an input function for the population pharmacokinetic (PK) model described in this paper. It was hypothesized that with the model for tablet GI transit and the developed PK model realistic predictions of plasma concentrations for formulations only studied *in vitro* could be made. The incorporation of biological variability in pH along the GI tract, between subject variability (BSV) in tablet GI transit, drug release and absorption processes all contributes to a prediction of the expected variability in plasma concentration *vs.* time profiles.

MATERIALS AND METHODS

Clinical Studies

Observations from three different clinical studies with AZD0837 were used for model building (Table I, Study 1–3). A fourth clinical study investigating three formulations for which no clinical data had been included in the model

Table I Clinical Study Cohorts Included in the Analysis

Study	Observations	Demography	Treatment	# subjects
1 (D1250C00018) MMM study	*In vivo* drug release, Tablet GI-position, Plasma concentration of AZD0837	All caucasian male, Age: 26–35 years (mean 31), BMI: 25.2–28.6 kg/m^2 (mean 26.5)	200 mg tablet, fasting adm.	6[a]
			200 mg tablet, fed adm.	6[a]
			200 mg tablet, adm. followed by food	6[a]
2 (D1250C00009) ADME study	Plasma concentration of AZD0837	All caucasian male, Age: 35–47 years (mean 41), BMI: 24.1–29.0 kg/m^2 (mean 26.0)	30 mg intravenous infusion (30 min)	10[a]
			240 mg oral solution, fasting adm.	10[a]
3 (D1250C00003) Bioperm® study	Plasma concentration of AZD0837	All caucasian male, Age: 20–29 years (mean 25), BMI: 19.5–26.5 kg/m^2 (mean 23.6)	50 mg bolus (~10 min) dose in colon	5
			50 mg infusion (2 h) in colon	7
4 (D1250C00004) IVIVC study	Plasma concentration of AZD0837	All caucasian male, Age: 20–40 years (mean 26), BMI: 19.5–26.8 kg/m^2 (mean 23.5 kg/m^2)	100 mg tablet A, fasting adm.	6
			100 mg tablet B, fasting adm.	6
			100 mg tablet B, fed adm.	6 (5)[b]
			100 mg tablet C, fasting adm.	6

[a] Cross-over-study

[b] 1 subject dropped out of study

building was utilized as an external validate dataset (Table I, Study 4). Plasma samples of AZD0837 were frequently collected up until or beyond 24 h after dose intake. Measurement of drug concentration was obtained using liquid chromatography-mass spectrometry (LC-MS) (10). The limit of quantification (LOQ) for plasma drug concentration was 10 nmol/l.

Magnetic Marker Monitoring

Described in detail in the accompanying article *A semi-mechanistic modeling strategy to link in vitro and in vivo drug release for modified release formulations* (12).

Bioperm® Colon Intubation

The Bioperm® technique (8) was utilized for administration of AZD0837 in colon. Administration was given in the form of a bolus dose (5–8 min infusion) and a 2 h infusion. To prepare for dosing via the Bioperm capsule the subjects swallowed the capsule in the morning on day 1 and in the morning of day 2 the drug was to be administered. The localization of the capsule in the gastrointestinal tract was monitored by the length of catheter swallowed and verified by an abdominal X-ray. The aim was to administer drug preferably in the ascending colon but also administrations in caecum and transverse colon were performed. In several cases the administration was not carried out since the capsule had either not reached or passed the designated place of administration. In total 18 subjects were randomized in the study but only 12 successful colon administrations were performed. The bolus dose and the 2-hour infusion of 50 mg AZD0837 solution in colon were administered with the subject in a horizontal position.

Model Building

Software

Data analysis was performed with a non-linear mixed effects approach as implemented in the NONMEM software version 7.1.2 (13), run on a Linux cluster with a Red Hat 9 operating system using OpenMosix and a G77 Fortran compiler. First order conditional estimation method (FOCE) with interaction and the ADVAN6 (general nonlinear kinetics) subroutine was applied for parameter estimation, standard errors for parameter estimates (covariance variance matrix) was obtained with importance sampling. The final NONMEM control file can be provided upon request.

The PsN toolkit version 3.2.7 (14,15) was used in conjunction with NONMEM for atomization and post processing purposes. The Xpose 4.3.0 (16,17) package in R (18) was used for graphical diagnostics.

Distribution and Elimination

Plasma concentration data from study 2 were initially modeled separately to establish a suitable model for characterization of AZD0837 disposition. Absorption was described with a lag-time and a first order absorption rate constant in order to include also data following administration of oral solution. A model with three distribution compartments and a hypothetical liver compartment was found to describe the disposition sufficiently. Renal CL had previously been established to be approximately 5% of total CL (10) and was fixed to that value (0.78 l/h) for all subjects. This was done to facilitate the implementation of a hypothetical liver compartment (19) describing hepatic elimination including first passage loss (Fig. 1, Eq. 3). The concept with a hypothetical liver compartment has been successfully applied several times before to separate hepatic first passage loss from other sources of loss during the absorption (primarily gut wall metabolism and/or incomplete absorption from the gut lumen) (6,20,21). The hepatic extraction ratio (E_H) was estimated with a logit-transformation (Eq. 1) to restrict estimates between 0 and 1. Allometric scaling was applied *a priori* to the hepatic blood flow (Eq. 2) and all volumes (Eq. 4) and inter-compartment clearances (Eq. 5) (22). The typical liver volume of a 70 kg person was assumed to be 1 l and the liver blood flow 90 l/h. The blood to plasma concentration ratio (Cb/Cp) for AZD0837 was set to 1.65 (AstraZeneca in house data).

$$E_H = \frac{e^{\left(\ln\left(\theta_{E_H}/\left(1-\theta_{E_H}\right)\right)+\eta_{E_H}\right)}}{1+e^{\left(\ln\left(\theta_{E_H}/\left(1-\theta_{E_H}\right)\right)+\eta_{E_H}\right)}} \tag{1}$$

$$Q_H = 3.5 \cdot Bodyweight^{0.75} \tag{2}$$

$$CL_H = E_H \cdot Q_H \cdot \frac{C_B}{C_P}. \tag{3}$$

$$V = V \cdot \frac{Bodyweight}{70} \cdot e^{\eta_V} \tag{4}$$

$$Q = Q \cdot \frac{Bodyweight^{0.75}}{70^{0.75}} \cdot e^{\eta_Q} \tag{5}$$

Rate and Extent of Absorption

Drug model describing drug release rate has been described in detail elsewhere (12), the substance released from the tablet was directed into GI compartments representing the different observed GI positions based on the time varying covariate of observed GI position. The GI position of the

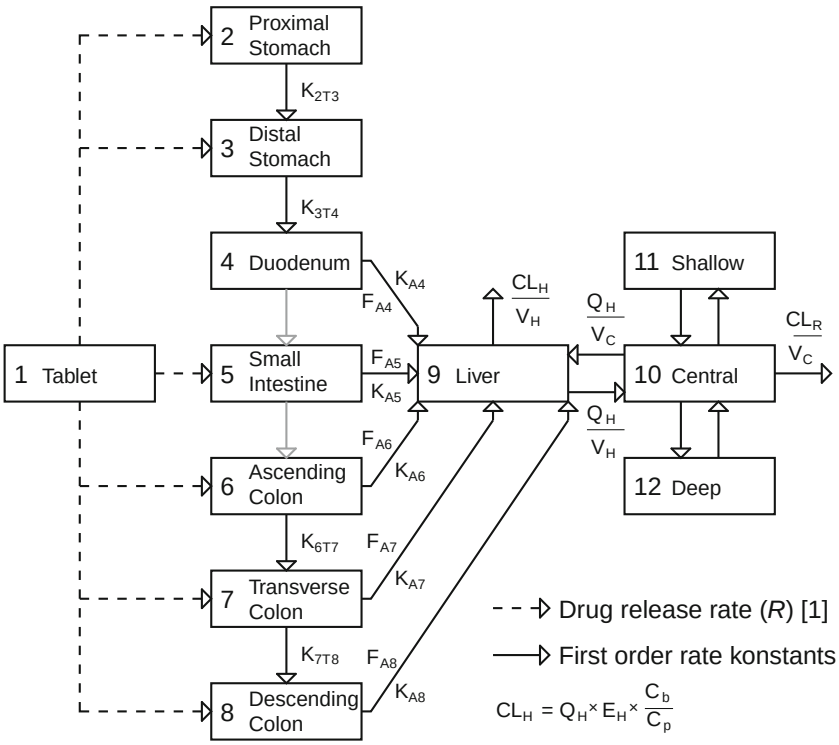

Fig. 1 Compartmental model structure applied to drug release, absorption and disposition of AZD0837. Compartment 1 represents the amount of drug present in the remaining tablet. Drug model describing drug release rate (*i.e.* elimination from the tablet compartment) has been described in detail elsewhere (12), the substance released from the tablet is directed into GI compartments 2–8 depending on the observed tablet position. Gastric emptying from the stomach compartments (comp. 2 and 3) are governed by first order rate constants (K_{2T3}, K_{3T4}), from duodenum drug can be absorbed over the gut wall via first order absorption rate constant (K_{A4}). The extent of absorption is limited by the fraction absorbed over the gut wall (F_{A4}). Since absorption rate was found to be rapid both from duodenum and lower parts of the small intestine no significant downstream mass transfer could be estimated for these compartments. Potential differences in rate and extent of absorption along the GI tract were assessed by investigating the benefit with separate K_A (K_{A4}-K_{A8}) and F_A (F_{A4}-F_{A8}) for the different GI compartments. Compartment 9 is a semi-physiological representation of the liver with a fixed volume (V_H = 0.0143 l/kg). Hepatic elimination is governed by allometrically scaled liver blood flow (Q_H), the blood plasma concentration ratio (C_b/C_p) and the estimated hepatic extraction ratio (E_H). A relatively small renal CL (CL_R) was assumed to be 0.78 l/h for all subjects. The systemic distribution of AZD0837 is described by a central observation compartment (comp. 10) and two peripheral compartments (comp. 11 and 12).

tablet was typically monitored continuously for 10 min followed by a 20 min period without observations. Transitions between the different GI positions frequently occurred between the monitoring sessions. This was handled by setting the unknown time of transit to halfway between the surrounding observations. It was assumed that there was no absorption from the stomach. The transport of released drug substance from the proximal stomach to the distal stomach and from the distal stomach further down into duodenum was described with two separate first order rate constants (K_{2T3} and K_{3T4}). An accelerating factor (+5 h^{-1}) was added to these gastric emptying constants at the time of tablet transit. This approach has been applied and scrutinized more thoroughly in previous work (6). This effect was thought to be the consequence of high concentration of released drug substance in the proximity of the tablet that is emptied simultaneous with the tablet. Duodenum was not among the GI regions characterized in the tablet position data. It is known from previous work (23) that solid dosage forms pass through duodenum very rapidly (<5 min) and therefore no significant amount of drug is released there. However it was evident from the early modeling attempts that the extent of absorption was significantly different for substance released in the stomach compared to substance released in the small intestine. This generated the hypothesis that rate and extent of absorption could be significantly different in the upper parts of the small intestine (*i.e.* duodenum). For this reason a duodenum compartment was implemented in-between the distal stomach and the small intestine. Separate rates (K_A) and extents (F_A) of absorption were investigated for the different GI positions duodenum, small intestine, ascending colon, transverse colon and distal colon (sigmoidal colon was treated as similar to distal colon due to very limited data). The rate of absorption in duodenum was found to be fast and not easily separated from rate of gastric emptying. It was therefore fixed to 30 h^{-1} indicating almost instantaneous absorption of drug emptied from the stomach. All other absorption related parameters were estimated simultaneous for all data with parameters for drug disposition

fixed to estimates from the separate analysis of study 2. The cross-over design of study 1 and 2 was respected and between subject variability was estimated (when feasible) assuming a log-normal distribution. For practical reasons no effort was made to characterize between occasion variability. Fraction absorbed for the different GI regions was estimated with logit-transformed parameters to restrict all estimates between 0 and 1.

Model Evaluation

VPC and pcVPC (24) were used for internal validation of the final model. These were based on 500 simulated replicates of the study data. VPCs were created for each treatment arm separately. These VPCs focused on the median prediction since each treatment arm included relatively few observations. The pvcVPC differ from the traditional VPC in that both observations and predictions are normalized to the median population prediction and median predicted between subject variability. This allowed the random between-subject-variability to be assessed by comparing observed and predicted 5th and 95th percentiles in a pooled pvcVPC across different treatments.

The final model together with the drug release model and tablet movement model described separately (12) was used to simulate an external validation dataset (study 4). The external dataset includes administration of three different formulations for which no clinical data had previously been obtained. The *in-vitro* drug release for these formulations was characterized with the same model that was applied to the formulation used in the MMM study. The model describing tablet GI transit was initially used to simulate tablet GI transit profiles. The GI transit profiles were subsequently used as a covariate for simulations with the drug release model and the absorption model. 500 replicates of the validation dataset were simulated and each summarized with median plasma concentration *versus* time for each treatment. Based on the 500 predicted median plasma concentration profiles a non-parametric 95% confidence interval for the median plasma concentration was calculated and presented graphically together with the observed median *versus* time.

RESULTS

Figure 1 is a schematic representation of the final model structure for GI tract distribution of the released drug substance, absorption and disposition of AZD0837. Parameter estimates and their imprecision are reported in Table II. Individual model fit to observed plasma concentrations following tablet administration and local colon administration is presented in Fig. 2. Visual predictive checks for internal validation are presented in Fig. 3 (study 1) and Fig. 4 (study 2 and 3). External validation by predictions of new ER formulations is presented in Fig. 5.

Table II Typical Parameter Estimates, Between Subject Variability (BSV) and Associated Relative Standard Errors (RSE)

Parameter (Unit)	Estimate (RSE,%)	% BSV (RSE,%)
E_H	0.16 (7.2)	11 (56)
V_C (l)	7.3 (22)	9.7 (130)
$Q_{shallow}$ (l/h)	17 (11)	
$V_{shallow}$ (l)	8.3 (18)	
Q_{deep} (l/h)	1.0 (53)	
V_{deep} (l)	2.6 (13)	
K_{2T3} (h^{-1})	0.63 (13)	
$K_{3T4\ fasting}$ (h^{-1})	4.2 (1.4)	126 (13)
$K_{3T4\ fed}$ (h^{-1})	1.3 (5.5)	22 (59)
K_{6T7} & K_{7T8} (h^{-1})	0.23 (53)	
K_{A4} (h^{-1})	30 (fix)	
K_{A5} (h^{-1})	3.3 (15)	
K_{A6} (h^{-1})	0.20 (7.4)	57 (164)
K_{A7} & K_{A8} (h^{-1})	0.16 (14)	71 (29)
F_{A4}	0.70 (2.8)	15 (57)
F_{A5}	0.25 (10)	59 (61)
F_{A6}	0.70 (7.2)	18 (51)
F_{A7} & F_{A8}	0.48 (65)	56 (25)
RUV[a] tablet (%)	23 (10)	
RUV[a] tablet (nmol/l)	32 (13)	
RUV[a] other (%)	15 (12)	
RUV[a] other (nmol/l)	12 (59)	

[a] Residual unexplained variability separated for tablet treatment and other treatments

A three compartment disposition model with a hypothetical liver compartment and allometric scaling was used to describe the distribution and elimination of AZD0837. Between subject variability (BSV) was characterized for hepatic extraction ratio (E_H) and central volume of distribution (V_C). For other disposition parameters the between subject variability was found to be negligible. The estimated BSV was approximately 10% for both E_H and V_C.

Rate of gastric emptying (K_{3T4}) was slower and less variable under fed than fasting conditions. Rate and extent of absorption was found to be significantly higher for substance released in the stomach and absorbed in duodenum compared to substance released in the main part of the small intestine. The fraction absorbed over the gut wall in the duodenum was 70% compared to 25% in the rest of the small intestine. The typical rate of absorption in duodenum was also significantly faster than the 3.3 h^{-1} estimated for small intestine. The exact rate of absorption in duodenum could not be estimated since it was hard to distinguish from rate of gastric emptying (K_{3T4}). The rate of absorption in the duodenum was therefore assumed to be

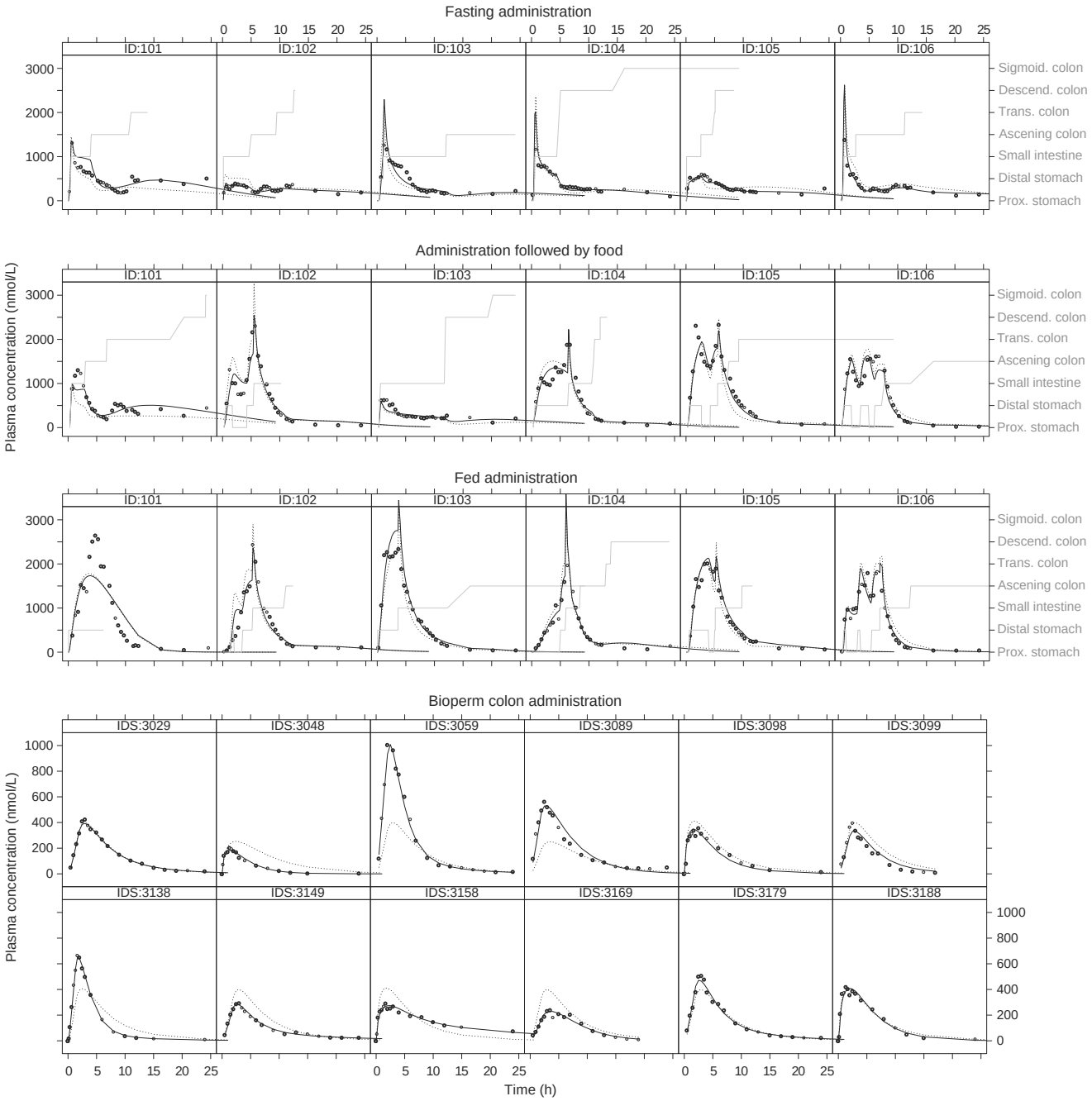

Fig. 2 Individual plasma concentrations *versus* time (open circles), population typical (dotted line) and individual model prediction (solid line) for AZD0837 following administration under different conditions. The gastro intestinal tablet position is indicated by a gray line represented on a secondary y-axis (left).

instantaneous ($K_{A4}=30\ h^{-1}$). In ascending colon the extent of absorption was similar to that in duodenum but the rate of absorption was considerably slower, $K_{A6}=0.2\ h^{-1}$. Rate of absorption was even slower and less complete in the lower parts of colon. Neither rate nor extent of absorption was found to be significantly different between transverse and descending colon. The rapid absorption in all of small intestine made distribution of released drug substance between duodenum, small intestine and ascending colon negligible and hence not possible to characterize. However the slow rate of absorption in colon made it possible to characterize the rate of distribution between ascending and transverse colon (K_{6T7}).

The residual unexplained variability was described with a combined additive and proportional residual error model. The residual error magnitude was found to be somewhat higher for observations following tablet administration compared to other means of administration (see Table II).

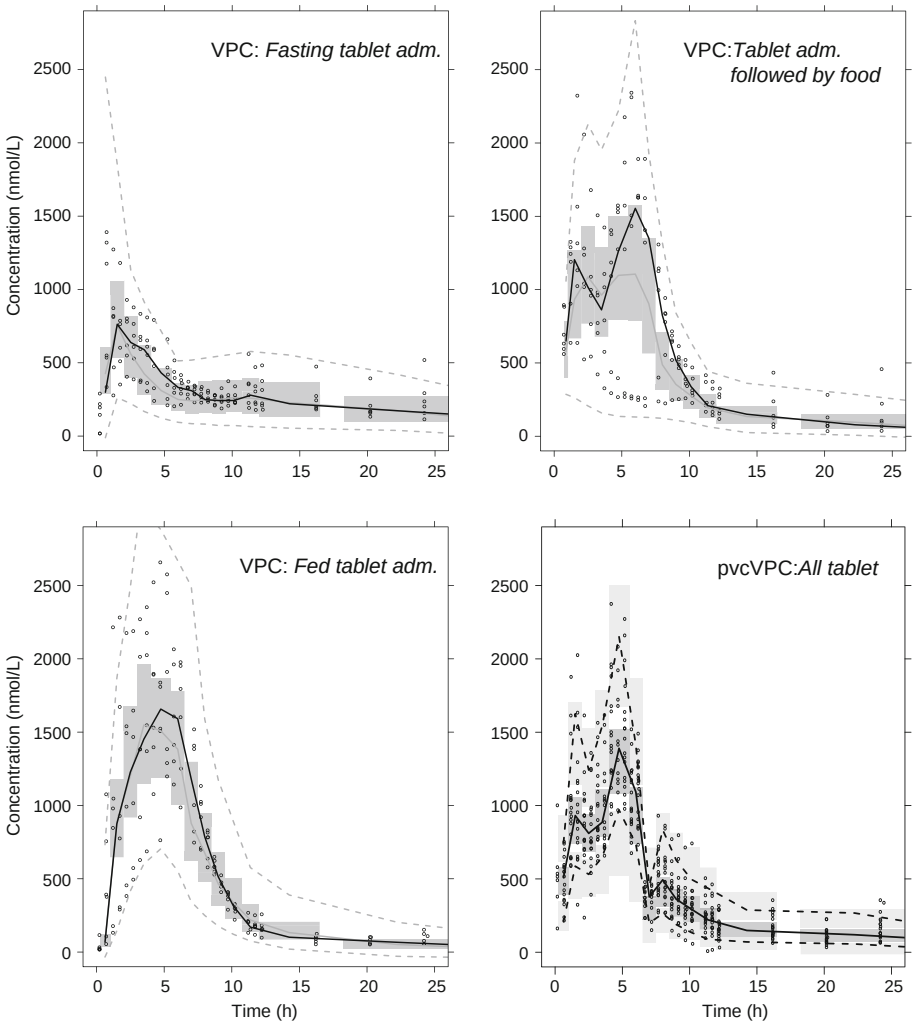

Fig. 3 Simulation based diagnostics of AZD0837 plasma concentration following tablet administration under different conditions for study 1. VPCs: observed plasma concentrations (open circles), observed median (solid black line), predicted non-parametric 95% confidence interval for the median (gray field) and 90% prediction interval (dashed gray lines). pvcVPC: prediction and variance corrected plasma concentrations (open circles), observed median (solid black line), predicted non-parametric 95% confidence interval for the median (dark gray field), observed 90% inter percentile range (dashed black lines) and corresponding 95% confidence interval (light gray fields).

The internal validation with VPC and pvcVPC demonstrates a good description of plasma concentrations across all routes of administration (Figs. 3 and 4). The external validation (Fig. 5) demonstrated good agreement between model predictions and observed plasma concentrations for formulation A. An overall acceptable prediction was also seen for formulation B (fed and fasting) and C but with a possible deviation indicated around 5–8 h after dose intake. Approximately 2–3 subjects indicated an increase in plasma concentration during this interval that was not predicted by the model.

DISCUSSION

Most of the large variability seen in plasma concentration profiles after tablet administration could be attributed to the tablet GI transit. The GI position had an important effect on drug release (12) but also on rate and extent of absorption. In the pvcVPC (Fig. 3, lower right hand panel) all observations and predictions are normalized based on the model prediction. The result is that the between subject variability originating from differences in independent model variables, primarily tablet GI transit but also to less extent body weight, is removed. The variability indicated in the pvcVPC is hence the variability that cannot be attributed to differences in tablet GI transit pattern and body weight. The good agreement between the 5th, 95th and median percentiles and their corresponding model predicted confidence intervals indicate a good model description of also the unexplained between subject variability.

The only effect seen of concomitant food intake apart from the effect on tablet GI transit (12) was a lower rate of gastric emptying under fed conditions. The estimated rate of distribution of released drug substance from proximal to distal stomach and gastric emptying was somewhat higher (20–50%) but following the same pattern as previously established for felodipine ER formulation (6). This can be a consequence of differences in how well initial drug release was described in the two models.

The assumption of different absorption characteristics in duodenum and the rest of small intestine was critical for

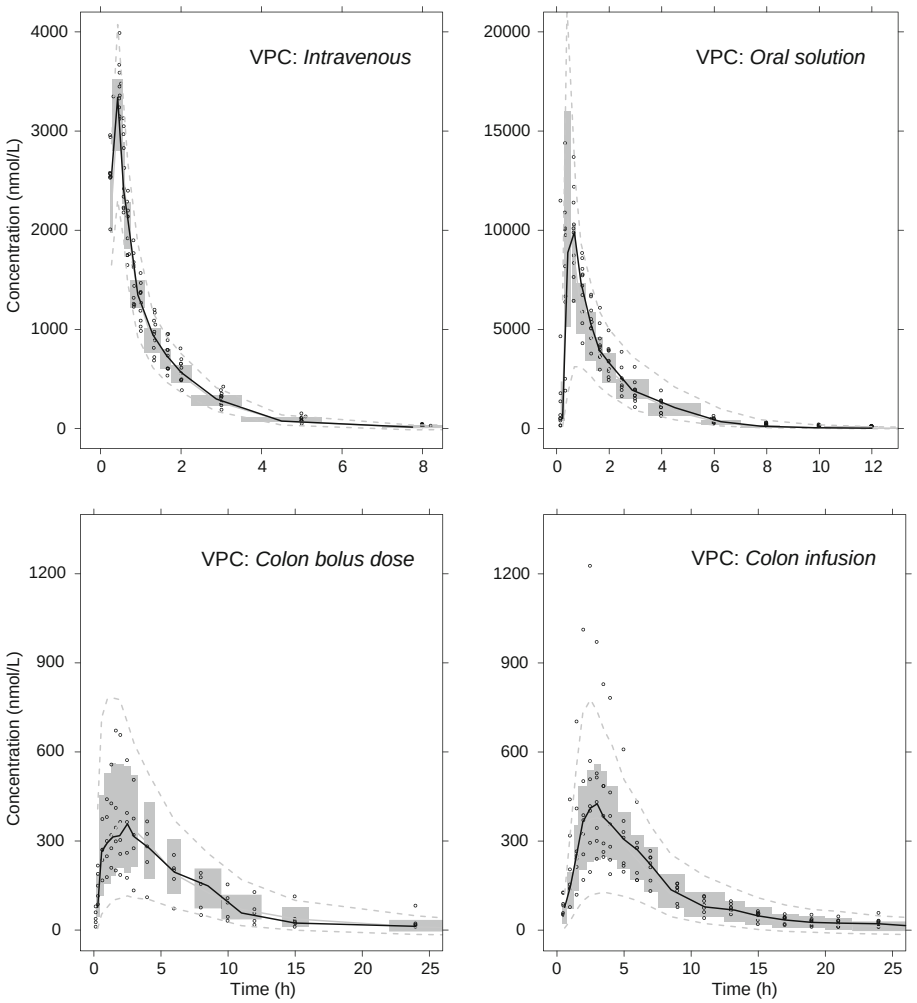

Fig. 4 Simulation based diagnostics of AZD0837 plasma concentration following different routes of administration for study 2 and 3. VPCs: observed plasma concentrations (open circles), observed median (solid black line), predicted non-parametric 95% confidence interval for the median (gray field) and 90% prediction interval (dashed gray lines).

achieving a satisfactory model fit. It was first assumed that only the rate of absorption was different and that this could be due to lower solubility at higher pH in small intestine compared to the stomach and duodenum. However, this assumption resulted in a relatively poor fit to the data and an unrealistically large between subject variability in rate of absorption for small intestine. One possible reason for the estimated low bioavailability over the small intestine gut wall could be differences in expression of metabolizing enzymes along the GI tract. A higher enzyme activity in the small intestine that increases the extraction of drug during absorption would result in a lower systemic bioavailability. *In vitro* assays indicate that AZD0837 are predominantly metabolized by CYP3A4 and CYP2J2. These enzymes are both known to be responsible for gut-wall metabolism (25). The expression of CYP3A4 has been documented to be high in the small intestine but to a much lower extent present in the stomach and colon (26). A rather substantial between subject variability has also been documented for the expression of CYP3A4 along the GI tract. CYP2J2 are on the other hand expressed fairly homogenously throughout the GI tract and with a low between subject variability (27). The estimated high bioavailability from colon is in good agreement with the low expression of CYP3A4 in that region. The literature points towards high expression of CYP3A4 in duodenum in a way that is in poor agreement with the estimated high bioavailability for this compartment. This could either be due to that the literature observations does not reflect the very early parts of duodenum or that the duodenum compartment implemented in the model actually represents absorption from the distal stomach. These two competing hypotheses cannot with certainty be discriminated between with the data at hand. To instead assume absorption from the distal stomach would alter the interpretation but not in any important way alter the predictions.

The amount of substance that passes out unchanged in feces was not measured and not estimated in the model. This might be contributing factor to the relatively high variability and on average slightly lower fraction absorbed from terminal parts of colon (transverse, descending and sigmoidal colon). Without measurements of amount of substance found in feces it will always be difficult to with

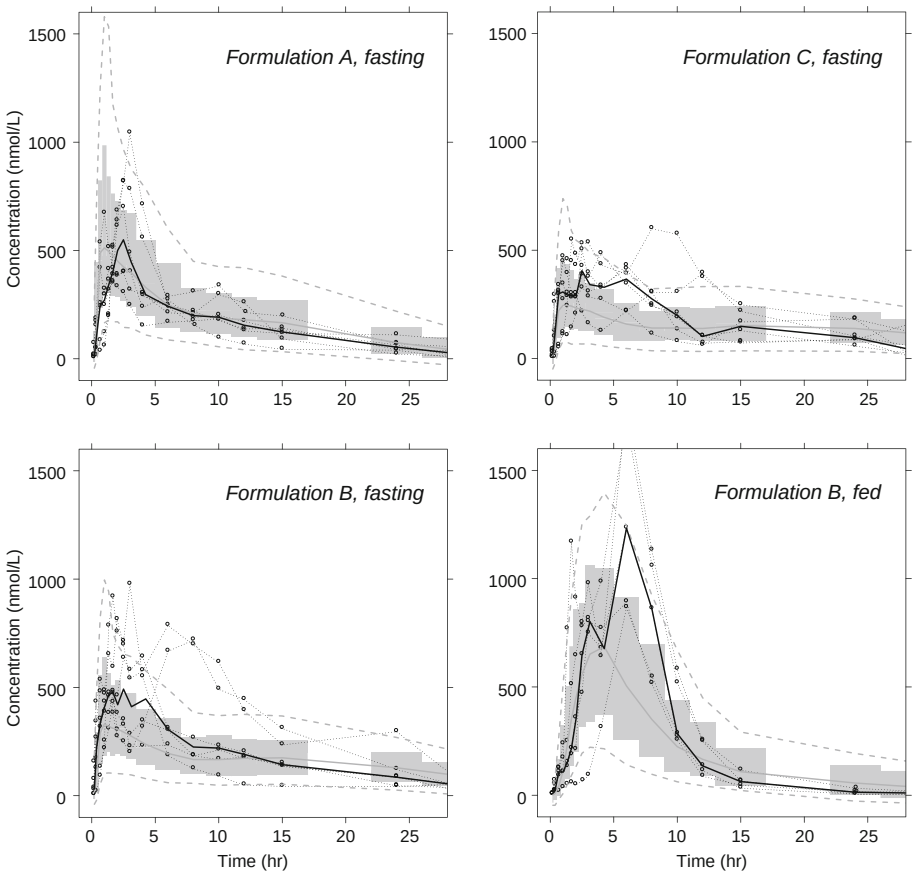

Fig. 5 Predictions of external validation dataset, study 4. Observed plasma concentrations (open black circles) connected with dotted black lines, observed median (solid black line), predicted non-parametric 95% confidence interval for the median (gray field) and 90% prediction interval (dashed gray lines).

certainty separate this loss from other sources of loss (*e.g.* gut wall metabolism).

Although the sample size of only 6 individuals per cohort in study 4 is too little to demonstrate a really convincing case of the quality for IVIVC, the prediction of external validation data-set was largely successful. The vast majority of the observations fall within the 90% prediction interval for each treatment arm and the central trend, represented by the median plasma concentration, is reasonably well predicted. Following fasting administration of formulation B and C, two subjects demonstrate a plasma concentration peak later than 5 h after dose intake. This pattern was unexpected for administration to fasting subjects and looked more similar to that anticipated when the tablet is taken together with food. Hence a delayed gastric emptying of the tablet could be an explanation for this pattern. Another explanation could be a fast drug release in colon for these subjects. For the fed administration of formulation B, all subjects demonstrate a plasma concentration peak at 6 h post dose. This is slightly later than what is predicted based on the model. This might be due to a sub optimal description of gastric emptying of tablets when administered together with food. The current Markov-model describes gastric emptying as a "constant hazard" where the food effect does not wear off with time (12). A model that more accurately capture the gastric emptying pattern could improve prospective predictions. However, the sample size of each individual MMM or gamma scintigraphy study is typically too small to support development of a more sophisticated model. This highlights the need for a meta-analysis of tablet GI transit data across several studies.

The model applied in this article is built with the aim of being parsimonious but able to explain oral absorption of AZD0837 when given in the form of an ER tablet. This is primarily a "top down approach" where clinical information on plasma concentration, *in vivo* drug release and GI position informs us about the absorption properties along the GI tract. There is also a "bottom up element" to the approach in that the drug release is characterized *in vitro* but incorporation of more *in vitro* and physiological prior information may be beneficial. A combination of approaches, "bottoms up and top down", is ideal to obtain a better understanding of oral absorption in particular and pharmacokinetic characteristics in general. There are, however, numerous practical problems to overcome in this type of approach and no perfect solutions to these limitations are available at the moment.

In this article, AZD0837 has served as an example compound to demonstrate a novel approach for *in vitro* to *in vivo* predictions. Robust *in vitro* to *in vivo* predictions are

undisputedly of great value in the development of new formulations. Furthermore the approach offers, yet unexplored, opportunities to support biowaiver applications to regulatory agencies. The suggested approach could offer the possibility to establish IVIVC in cases when the standard approach described in regulatory guidelines is inadequate (28). None of the formulations investigated in this manuscript are intended for further clinical usage. The lessons from these studies have guided the formulation development towards formulations demonstrating less food interaction and generally lower between subject variability. Plasma concentrations of AZD0837, which is a prodrug, have in the present model been used to describe the *in vivo* disposition. It is possible to extend the model to also include plasma concentrations of the active form (AR-H067637) and pharmacodynamic variables related to its anticoagulant effect. Although this will increase the complexity of the model, it may add value to better evaluate the clinical relevance of variability in release profile and *in vivo* absorption for the ER formulation.

CONCLUSION

Varying absorption properties for AZD0837 along the GI tract were characterized with a semi mechanistic PK model. By combining this model and models describing *in vitro*/*in vivo* drug release and tablet GI transit, prospective predictions of plasma concentrations following administration of newly developed ER formulations could be made based on *in vitro* release profile. Comparison of model predictions, including inter-individual variability, and actual observed plasma concentrations showed an overall satisfactory predictive performance. This modeling and simulation approach could be used for guiding formulation development and establishing acceptable deviations of *in vitro* performance for modified release formulations.

ACKNOWLEDGMENTS & DISCLOSURES

All studies described in the manuscript were sponsored by AstraZeneca. Martin Bergstrand and Mats O. Karlsson have received research funding from AstraZeneca. Erik Söderlind and Ulf G. Eriksson are employees of AstraZeneca.

REFERENCES

1. Casey DL, Beihn RM, Digenis GA, Shambhu MB. Method for monitoring hard gelatin capsule disintegration times in humans using external scintigraphy. J Pharm Sci. 1976;65(9):1412–3.
2. Digenis GA, Sandefer EP, Page RC, Doll WJ. Gamma scintigraphy: An evolving technology in pharmaceutical formulation development - Part 1. Pharm Sci Technol Today. 1998;1(3):100–7.
3. Digenis GA, Sandefer EP, Page RC, Doll WJ. Gamma scintigraphy: An evolving technology in pharmaceutical formulation development - Part 2. Pharm Sci Technol Today. 1998;1(4):160–5.
4. Weitschies W, Blume H, Mönnikes H. Magnetic marker monitoring: High resolution real-time tracking of oral solid dosage forms in the gastrointestinal tract. Eur J Pharm Biopharm. 2009;74(1):93–101.
5. Weitschies W, Kosch O, Mönnikes H, Trahms L. Magnetic Marker Monitoring: An application of biomagnetic measurement instrumentation and principles for the determination of the gastrointestinal behavior of magnetically marked solid dosage forms. Adv Drug Deliv Rev. 2005;57(8):1210–22.
6. Bergstrand M, Söderlind E, Weitschies W, Karlsson MO. Mechanistic modeling of a magnetic marker monitoring study linking gastrointestinal tablet transit, *in vivo* drug release, and pharmacokinetics. Clin Pharmacol Ther. 2009 Jul;86(1):77–83.
7. Sutton SC. The use of gastrointestinal intubation studies for controlled release development. Br J Clin Pharmacol. 2009;68(3):342–54.
8. Nyberg L, Mansson W, Abrahamsson B, Seidegard J, Borga O. A convenient method for local drug administration at predefined sites in the entire gastrointestinal tract: experiences from 13 phase I studies. Eur J Pharm Sci. 2007 Apr;30(5):432–40.
9. Olsson SB, Rasmussen LH, Tveit A, Jensen E, Wessman P, Panfilov S, *et al.* Safety and tolerability of an immediate-release formulation of the oral direct thrombin inhibitor AZD0837 in the prevention of stroke and systemic embolism in patients with atrial fibrillation. Thromb Haemost. 2010;103(3):604–12.
10. Johansson S, Cullberg M, Eriksson UG, Elg M, Dunér K, Jensen E, *et al.* Single-dose pharmacokinetics, pharmacodynamics and safety of AZD0837, a novel oral direct thrombin inhibitor, in young healthy male subjects. Int J Clin Pharmacol Ther. 2011;49(4):258–67.
11. Lip GYH, Rasmussen LH, Olsson SB, Jensen EC, Persson AL, Eriksson U, *et al.* Oral direct thrombin inhibitor AZD0837 for the prevention of stroke and systemic embolism in patients with non-valvular atrial fibrillation: A randomized dose-guiding, safety, and tolerability study of four doses of AZD0837 *vs.* vitamin K antagonists. Eur Heart J. 2009;30(23):2897–907.
12. Bergstrand M, Söderlind E, Eriksson UG, Weitschies W, Karlsson MO. A mechanistic model to link *in vitro* and *in vivo* drug release for modified release formulations. [unpublished companion manuscript].
13. Beal SL, Sheiner LB, Boeckmann AJ. NONMEM User's Guides. Ellicot City: MD: Icon Development Solutions; 1989–2006.
14. Lindbom L, Ribbing J, Jonsson EN. Perl-speaks-NONMEM (PsN)–a Perl module for NONMEM related programming. Comput Methods Programs Biomed. 2004 Aug;75(2):85–94.
15. Lindbom L, Pihlgren P, Jonsson EN. PsN-Toolkit–a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. Comput Methods Programs Biomed. 2005 Sep;79(3):241–57.
16. Jonsson EN, Karlsson MO. Xpose–an S-PLUS based population pharmacokinetic/pharmacodynamic model building aid for NONMEM. Comput Methods Programs Biomed. 1999 Jan;58(1):51–64.
17. Harling K, Ueckert S, Hooker AC, Jonsson EN, Karlsson MO. Xpose and Perl speaks NONMEM (PsN). PAGE 19 (2010) Abstr 1842 [wwwpage-meetingorg/?abstract=1842].
18. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing; 2009 [2009-12-30]; Available from: http://www.R-project.org.
19. Gordi T, Xie R, Huong NV, Huong DX, Karlsson MO, Ashton M. A semiphysiological pharmacokinetic model for artemisinin in

healthy subjects incorporating autoinduction of metabolism and saturable first-pass hepatic extraction. Br J Clin Pharmacol. 2005 Feb;59(2):189–98.
20. Gordi T, Xie R, Huong NV, Huong DX, Karlsson MO, Ashton M. A semiphysiological pharmacokinetic model for artemisinin in healthy subjects incorporating autoinduction of metabolism and saturable first-pass hepatic extraction. Br J Clin Pharmacol. 2005;59(2):189–98.
21. Magnusson MO, Dahl ML, Cederberg J, Karlsson MO, Sandström R. Pharmacodynamics of carbamazepine-mediated induction of CYP3A4, CYP1A2, and Pgp as assessed by probe substrates midazolam, caffeine, and digoxin. Clin Pharmacol Ther. 2008;84(1):52–62.
22. Holford NH. A size standard for pharmacokinetics. Clin Pharmacokinet. 1996 May;30(5):329–32.
23. Weitschies W, Cardini D, Karaus M, Trahms L, Semmler W. Magnetic marker monitoring of esophageal, gastric and duodenal transit of non-disintegrating capsules. Pharmazie. 1999 Jun;54 (6):426–30.
24. Bergstrand M, Hooker AC, Wallin JE, Karlsson MO. Prediction-Corrected Visual Predictive Checks for Diagnosing Nonlinear Mixed-Effects Models. AAPS J. 2011 in press.
25. Thelen K, Dressman JB. Cytochrome P450-mediated metabolism in the human gut wall. J Pharm Pharmacol. 2009;61(5): 541–58.
26. Paine MF, Khalighi M, Fisher JM, Shen DD, Kunze KL, Marsh CL, *et al.* Characterization of interintestinal and intraintestinal variations in human CYP3A-dependent metabolism. J Pharmacol Exp Ther. 1997 Dec;283(3):1552–62.
27. Zeldin DC, Foley J, Goldsworthy SM, Cook ME, Boyle JE, Ma J, *et al.* CYP2J subfamily cytochrome P450s in the gastrointestinal tract: Expression, localization, and potential functional significance. Mol Pharmacol. 1997;51(6):931–43.
28. Jantratid E, De Maio V, Ronda E, Mattavelli V, Vertzoni M, Dressman JB. Application of biorelevant dissolution tests to the prediction of *in vivo* performance of diclofenac sodium from an oral modified-release pellet dosage form. Eur J Pharm Sci. 2009;37(3–4):434–41.