Estimating the Variability in Fraction Absorbed as a Paradigm for Informing Formulation Development in Early Clinical Drug Development

Sarit Cohen Rabbie[1], Paul D. Martin[2], Talia Flanagan[2], Abdul W. Basit [1] and Joseph F. Standing [3]

[1]Department of Pharmaceutics, UCL School of Pharmacy, University College London, London, UK

[2]AstraZeneca, Alderley Park, Cheshire, UK

[3]UCL Institute of Child Health, University College London, London, UK

Key words: Inter-subject variability, absorption, bioavailability, formulation, food effect.

Abbreviation:

F - Bioavailability

fa- fraction absorbed

fg -fraction passing the gut wall

fh -fraction escaping hepatic metabolism

GI –gastrointestinal

NLME- non linear mixed effect modelling

IR- immediate release

PR- prolonged release

ICH- international conference on harmonisation

GCP- good clinical practice

CLR – renal clearance

CLH- hepatic clearance

WT – weight

FQ- Liver blood flow

BPR- blood /plasma ratio

CLi- intrinsic clearance

LV- liver volume

ER- extraction ratio

FOCE- first order conditional estimation

PK- pharmacokinetics

OFV- objective function value

VPC – visual predictive check

PSN-perl speaks NONMEM

CV- coefficient of variation

Corresponding author: Sarit Cohen-Rabbie, sarit.rabbie@astrazeneca.com, Melbourne Science Park, Royston, Hertfordshire, SG8 6HB +44(0)7469400635

Abstract

Purpose: Inter-subject variability in oral drug absorption is usually reported using bioavailabilty, which has the components: fraction absorbed (fa), fraction passing the gut wall (fg) and fraction escaping hepatic metabolism (fh). In this study, we sought to separate the absorption (fa*fg) and elimination (fh) components of bioavailability to study variability of absorption and to investigate the effect of formulations, gastric pH and food on absorption variability.

Methods: Four compounds from the AstraZeneca database with a range of reported bioavailabilties (high, intermediate 1&2 and low) were selected. First, a disposition model using intravenous data was developed; Second, intrinsic clearance and hence hepatic extraction ratio was estimated based on the "well stirred" model; lastly, the oral data were included to enable estimation of fa*fg as a separate component to hepatic extraction. Population pharmacokinetic model fitting was undertaken with NONMEM v.7.2.

Results: The limiting step in absorption for intermediate 1 was dissolution rate and fa*fg variability increased under elevated gastric pH (15% vs. 38%, respectively). Absorption of solution formulation intermediate 2 increased by 17% in the presence of food but the prolonged release formulation's absorption didn't differ under fasted or fed state. Variability wasn't affected by food for both formulations (~30%). For the low bioavailable compound, variability decreased when formulated as a prolonged-release formulation (39% vs. 15%).

Conclusions: The method described here enables an exploration of drug absorption inter-subject variability using population pharmacokinetics. Implementation of such an approach may aid the formulation design process through a better understanding of the factors affecting oral drug absorption variability.

1. Introduction

The terms absorption and bioavailability (F) are often used interchangeably (1), though these are distinct concepts. Indeed, whereas bioavailability is defined as the fraction of an oral dose administered that reaches the general circulation or site of action, the fraction absorbed (f_a) is the fraction of a dose that enters the cellular space of enterocytes from the gut lumen. Two other parameters contribute to drug bioavailability: f_g, the fraction of drug entering the enterocytes that escapes first-pass gut wall metabolism and enters the portal vein; and f_h, the fraction of drug entering the liver that escapes first-pass hepatic metabolism and biliary secretion, thus entering the systemic circulation (Equation 1) (2).In this investigation, the term absorption shall refer to both the fraction absorbed and the fraction that escapes gut wall metabolism due to limitation in estimating f_g.

$$F = f_a * f_g * f_h$$

Equation 1: Oral bioavailability

All parameters mentioned in Equation 1 are sensitive to inter-subject differences (3,4). The factors which contribute to inter-subject variability in f_a are formulation aspects (namely, disintegration and particle size); physicochemical attributes of the drug (dissolution and solubility); and variation in GI physiology, including gastrointestinal (GI) tract functions as represented by pH changes, gastric emptying time and intestinal transit time varying with age, gender, and diseases. Other factors, including food, alcohol or concomitant medication use, may also affect the drug dissolution or GI function (5). f_g is sensitive to the abundance and the regional distribution of drug metabolizing enzymes (which could be influenced by genetics and diet), variation in blood flow to the gut, and disease states. Changes in the activity of drug metabolizing enzymes in the liver as a result of environmental substances or toxins as well as genetic makeup (expression level and polymorphism), can contribute to inter- subject variability in f_h. Other factors affecting hepatic clearance variability are related to age, ethnic groups and gender. The contribution of these factors together with inter-subject variability adds a layer of complexity to the situation *in vivo*, and hence an explicit mechanistic understanding is required. Focusing on f_a from oral administration specifically can improve understanding of the key causes of

low absorption and consequent variability in this parameter. In turn, understanding f_a and its associated inter-subject variability in the early stages of drug development provides an opportunity to understand absorption mechanism, optimise formulation performance by increasing drugs solubility or dissolution rate, and consequently to increase drug absorption. However, the effect of different formulations on inter-subject variability is usually not assessed at such an early stage in the clinical development process.

When analysing clinical pharmacokinetic data (as drug plasma concentrations), it is common to use non-linear mixed-effect modelling (NLME) – the so-called "population approach" (FDA Guidance for Industry Population Pharmacokinetics). The advantage of this modelling approach is the improvement in underlying effects in drug performance which is important in understanding variability in population (6).

One of the more difficult tasks for a modeller is to find an appropriate structural description of drug absorption, as the population pharmacokinetic modelling approach should be executed while taking into account the physicochemical properties of a drug, the physiology of the subject and the variability of all the different mechanisms of absorption. The traditional models used to describe the absorption process are simple and include a parameter describing the absorption rate (first or zero order absorption rate constant), bioavailability and usually a lag time parameter characterizing any potential absorption delay. Given the importance of characterizing absorption, more effort should be expended on developing these models.

In this study, the well-stirred model was used to separate f_h from f_a*f_g in place of bioavailability in order to gain a better understanding of inter-individual variability in absorption from different formulations in phase I/II clinical studies. The population approach allowed the determination of the magnitude of inter-subject (individuals) variability. The population pharmacokinetics of four compounds with different reported bioavailabilities (Table I) were tested by the simultaneous fitting of data from different drug formulations, including oral solution, immediate-release (IR) and prolonged-release (PR) formulations. In addition, inter-subject variability in absorption of these drugs was investigated in relation to food effect and gastric pH. We aimed to use these examples to show how

such an approach could be useful in formulation development and understanding the important factors affecting inter-subject variability in absorption in early stages of clinical development from clinical trials.

2. Methods

2.1. Data

Four compounds with low (AZD7009 developed for atrial fibrillation), intermediate 1 and 2 (AZD0865 and AZD1305 developed for gastroesophageal reflux disease and for treatment of atrial fibrillation, respectively) and high (AZ242-developed for diabetes mellitus) bioavailability were identified from the AstraZeneca clinical trials database. All datasets were phase I/II studies performed in healthy male volunteers, conducted in accordance with the Declaration of Helsinki, which were compliant with the International Conference on Harmonisation (ICH)/Good Clinical Practice (GCP) and regulatory requirements, and also the AstraZeneca policy on Bioethics. Compound selection was based on availability of intravenous data and differing physicochemical and pharmacokinetic properties. Physicochemical properties and pharmacokinetic parameters based on non-compartmental analysis for each compound are specified in Table I. In addition, the plasma vs. time profiles on log scale of the four compounds in the different formulations are presented in Figure 1. Inter-subject variability in oral drug absorption was investigated in relation to the effect of PR (prolonged release) formulation, gastric pH and food effect and for the low, intermediate 1 and intermediate 2 compounds, respectively. Clinical trials from phase I/II were incorporated in the analysis and f_a*f_g was estimated for the oral solution and different formulations under the mentioned conditions. The number of subjects, demographics (age and weight) and administered doses are listed in Table II.

2.2. Model building

Population pharmacokinetic model building was undertaken using NONMEM VII (V-12, Icon plc, http://www.iconplc.com/innovation/solutions/nonmem/). The process of finding the optimal model includes four major steps: model definition, model fit, model diagnostics and model evaluation. NONMEM is a tool for building mathematical model of this underlying process using several building blocks. The basic block is the structural model. An example of collected data includes the measurement of the plasma concentration over time. Inferences from the data are drawn and summarized in terms of estimated model parameters, such as drug clearance (CL). Another important component of the model is variability. Therefore, parameters of the model are treated as distributions, rather than single values. This is the second building block called "random effects (the measurements "noise"). In biological data, there are two sources of random variability which are quantified in mixed effect analysis: variability between different individuals – inter-individual variability (IIV) and residual variability (RV). Inter-individual variability is considered at the level of the model parameter and the residual variability is at the level of the observed data point and includes noise due to measurement error, erroneous data records, and changes in individual biology over time, or error due to model misspecification (Fisher/Shafer NONMEM Workshop Pharmacokinetic and Pharmacodynamic Analysis with NONMEM, Basic Concepts, 2007)

In this study, individual plasma concentration vs. time from different clinical trials of the same formulation were pooled to form a single dataset, with mass units expressed in nanomoles. Raw plots of plasma drug concentration vs. time were generated using R (Version R-3.1.1, available on http://www.r-project.org/) and inspected for possible trends in the structural models (Figure 1). Disposition of each compound was determined by modelling the intravenous data alone. One-, two-, three- and four-compartment models (Volume of distribution and clearance values of central and peripheral compartments) with CL split into the renal component (CLR) and hepatic clearance (CLH).

CLR was fixed according to unchanged urine excretion, and the hepatic component (CLH) estimated using the well-stirred liver model as follows:

Liver volume (LV) in Liters was associated to the subject weight as indicated by Noda *et al*. (7) in Equation 2:

$$LV = 0.05012 * WT^{0.78}$$

Equation 2: Liver volume based on publication from Noda et *al*. (7)

Liver blood flow (FQ) in males was reported as 50.4 L/h/L of liver volume. The blood/plasma ratio, measured in vitro (AstraZeneca in house data set), was used to take into account the total blood to total plasma drug concentration ratio (BPR-as fixed value) (Equation 3):

$$FQ = 50.4 * LV * BPR$$

Equation 3: Liver blood flow

Intrinsic clearance (CLi) was estimated parameter from the disposition model and was used to calculate the hepatic clearance (Equation 4):

$$CLH = \frac{FQ * CLi}{(FQ + CLi)}$$

Equation 4: Clearance hepatic calculation based on intrinsic clearance

Allometric weight scaling was added to renal clearance fixed effects *a priori*, standardized to a body weight of 70 kg according to the following relationships (8) (Equation 5 and Equation 6):

$$CL = CLH + CLR$$

Equation 5: Clearance calculation

$$CLR = CLR * ((WT)/70)^{0.75}$$

Equation 6: Renal clearance normalised by weight

The IV data were analysed with first-order conditional estimation (FOCE) plus interaction (inter-individual and residual variability). For the IV data analysis, the ADVAN7 TRANS1 (General Linear Model with Real Eigen value and estimation of Q and V PK parameters) subroutine in NONMEM was used.

Once an adequate structural model was identified, the disposition parameters and its associated inter subject variability were then fixed, on the assumption that absorption model mis-specification may unduely influence disposition parameters when data were pooled. Additional (e.g. oral) data for each formulation or condition (fast\fed) from different studies were pooled, and the absorption model was developed.

The extraction ratio (ER) was calculated based on the intrinsic hepatic clearance (CLi) and liver blood flow (FQ) and was further utilised to estimate f_a*f_g based on bioavailability (F1) NONMEM estimation (**Error! Reference source not found.**& **Error! Reference source not found.**):

$$ER = \frac{CLi}{(FQ + CLi)}$$

Equation 7: Enterohepatic circulation

$$F1 = fa * fg * (1 - ER)$$

Equation 8: Calculation of f_a*f_g based on the well stirred model

For the oral absorption modelling the ADVAN5 TRANS1- (linear general model and estimation of Q and V, PK parameters) NONMEM subroutine was used. Inter-individual variability for PK parameters was estimated using an exponential model (log normal model), except for the f_a*f_g, where a Logit transformation was used to ensure the individual estimate remained between 0 and 1. Mixed additive and proportional model was tested for residual error.

Lag-time or a discrete number of transit compartments were used to describe delays in absorption. Due to long runtimes when using ordinary differential equation solver methods (e.g. ADVAN6), for the transit model a stepwise search for the optimal number of transit compartments (n) was conducted based on the lowest OFV (Figure 2). Two typical run files of the disposition model and absorption model are provided as supplementary information.

2.3. Pharmacokinetic model evaluation and simulations

Model selection was achieved by use of the objective function value (OFV- an objective function value is the sum of squared deviations between the predictions and the observations. In NONMEM, the objective function is -2 times the log of the likelihood. A difference in objective function value of 3.84 is considered to be significant at $p<0.05$ with one degree of freedom, based on chi squared distribution)); successful covariance step; by examination of relative standard error values and goodness-of-fit plots. Xpose (version 4.0) and R software (Version 3.1.1) were used for the graphical goodness-of-fit analysis. A visual predictive check (VPC) was employed to characterize the model's simulation properties. The final model was used to simulate 500 new datasets, based on the design of the original data set. For each of the original data points, a 95% prediction interval was obtained by extracting the 2.5% and 97.5% percentiles of their simulated distributions. These were then plotted against the observations using PsN (Perl speaks NONMEM Version 3.5.3) and Xpose (version 4.0).

To estimate inter-subject variability, simulations using R in 1000 subjects were carried out to estimate variance from the model, the square root of the variance being the standard deviation (using Multivariate Normal Density and Random Deviates package to provide the density function and a random number generator for the multivariate normal distribution with mean equal to mean and covariance matrix sigma). The coefficient of variation (CV%) was then calculated by dividing the standard deviation by the mean value (Table IV). PsN was also used to run a nonparametric bootstrap of 200 iterations to provide unbiased estimates of the standard errors and the 95% confidence intervals of the estimated parameters (Figures 4A, B and C). The terms high and low variability refer to distributions that have high and low coefficients of variation, respectively. Typically, a coefficient of variation of a pharmacokinetic parameter of 10% or less is considered low, 25% is moderate, and above 40% is high (9).

3. Results

The best fit for the disposition model for all four compounds was achieved with a three compartment model. For high, intermediate 1, intermediate 2 and low bioavailability compounds, the OFV decreased significantly, when changing from two compartments model to a three compartments model. In addition, no significant improvement in fit with a four compartment model was observed for any compound. A successful covariance step was observed for all structural models. Shrinkage percentages are in the acceptable range (Table I, supplementary appendix). The disposition parameters of the final structural model for all four compounds are presented in Table III. Reasonable goodness-of-fit plots confirmed that the structural model adequately described the data (Figure 1, supplementary appendix).

After fixing the disposition parameters and adding the oral data, estimations of f_a*f_g and k_a (absorption rate constant) were carried out by adding an absorption compartment. Improvement in fit using a lag-time was tested for oral solution formulations for all compounds, and appeared to improve the fit. Adding transit compartment in the case of oral solution administration did not yield a significant decrease in OFV and therefore was not included in the final model. For all compounds in the solid forms formulations addition of transit compartment improved the model fit and were included in the final model.

The goodness-of-fit plots for all solution formulations and solid dosage forms formulation are provided in the supplementary appendix in Figures 2 and 3 (A, B and C), respectively, showing that the model fit in particular in early time points (which represent the absorption phase) is reasonably acceptable. The visual predictive check for all compounds, presented in Figure 3 (A, B, C and D) indicates that the final model was able to simulate data with a similar distribution to the observed data. The VPC is showing that the median, 5^{th}, 50^{th} and 95^{th} percentiles of the observations lie within the 95% CI of the model simulation. The nonparametric bootstrap of 200 iterations estimates of the standard errors and the 95% confidence intervals of f_a*f_g presented in Box plots graphs (Figure 4).

f_a*f_g and inter-subject variability estimations for the different formulations presented in Table IV. For the low bioavailability compound, f_a*f_g for the oral solution was estimated to be 33% with inter-subject variability of 39% which increased when the compound was formulated as prolonged release formulation (57%) and variability decreased to 15%. The effect of different forms of the active ingredient (salt and base forms) on the inter-subject variability in absorption was investigated for intermediate 1. f_a*f_g of the salt form increased compared to the base form although variability was similar for both forms. However, under elevated gastric pH, inter-subject variability in f_a*f_g increased for both forms. Food effect absorption was investigated for intermediate 2 compound when an oral solution and a PR formulation were given under fasted and fed conditions. Positive food effect was observed when oral solution was administered (increase of 16% in f_a*f_g) which diminished when the PR formulation was administered. Inter-subject variability was similar across all formulations under fed and fasted states (CV~30%).

3. Discussion

In this investigation, the "well stirred "model was successfully implemented in NONMEM analysis to focus on the drug absorption and its associated inter-subject variability, and not overall bioavailability. The high bioavailability compound was chosen as a control drug to confirm that the absorption values generated by NONMEM with the fitted "well-stirred" model equations are valid. The absorption rate constant was high for the oral solution, indicating that the compound is rapidly absorbed. 100% absorption was estimated for the oral solution with short lag time. A relatively low inter-individual variability was estimated for this compound (9%).

3.1. Effect of formulations on inter-subject variability (low bioavailability compound)

For the low bioavailability compound, the absorption rate was faster in the case of the solution compared to the PR formulation (3.2 and 0.04 h^{-1}, respectively), with higher variability in the solution absorption rate than in the solid dosage form. Low absorption was estimated for the oral solution and high variability whereas the PR formulation absorption increased to 60% and corresponded inter-subject variability decreased by more than half. The low absorption after oral solution administration (30%) indicates f_h is around 50%. Therefore, the low bioavailability in the case of the solution dosage form can be attributed to both absorption and hepatic elimination. Inter-subject variability in absorption is higher compared to the inter-subject variability in bioavailability (40% vs 26%), indicating absorption process might be responsible for major differences between subjects.

Considering the physicochemical properties of this compound (Table I), solubility or dissolution should not be the rate-limiting step, as in its given dose it is expected to be completely dissolved in the GI fluids. The increase in the absorption for the PR formulation might indicate a possible stability issue for the drug in the upper part of the gastrointestinal tract. Allowing for a low dissolution rate in the upper part of the gut will enable more of the drug to reach the lower parts of the gut, thus prolonging absorption. In addition, no gut wall metabolism is expected based on clinical trial data that showed that no effect on drug pharmacokinetics when co-administered with the P-gp inhibitor verapamil (AstraZeneca data file).

3.2. The effect of gastric pH on inter-subject variability in absorption (intermediate 1 bioavailability compound)

Based on the absorption estimations for the oral solution for intermediate 1 compound (60%), f_h estimated at around 90%, indicating low hepatic extraction; therefore, the medium bioavailability can be attributed to the decrease in absorption. Inter subject variability of the oral solution absorption was lower compared to the inter-subject variability in bioavailability, but still classified as medium inter-subject variability (22% vs. 15%). The absorption of the base form did not differ from the oral solution (~60%). 15% increase in the drug absorption for the salt form might indicate that the absorption is solubility/dissolution rate-limiting.

The intermediate 1 compound is a weak base therefore, its solubility would be highly dependent on the gastrointestinal pH, and drug precipitation might occur as a consequence of the pH increase from acidic in the stomach (especially in the fasted state) to near-neutral in the small intestine (10). It seems that the salt formulation managed to minimize precipitation, and yielded a super-saturated state for a longer period of time in order to allow longer absorption. To emphasise that, the absorption of the base and the salt forms decreased 4 and 2 folds respectively, at elevated gastric pH. At elevated gastric pH, the compound solubility in the gastric fluid is low, and almost all the drug would be emptied into the duodenum from the stomach in the undissolved form. Both the rate and extent of absorption are therefore limited by intestinal drug dissolution.

A separation of the fraction that escapes gut wall metabolism (f_g) from the fraction absorbed (f_a) was not made in this analysis. However, based on clinical studies where the compound was administered with grapefruit juice, and which did not seem to affect the pharmacokinetics of intermediate 1, this indicates that metabolism by CYP 3A4/3A5 in the gut is of minor importance for the pharmacokinetics of intermediate 1.

The inter-subject variability estimated herein was similar for all formulations around 15%, and increased under elevated gastric pH conditions. The increase in solubility of the drug using the salt

formation did not affect the inter-subject variability. The medium inter-subject variability might mask the increase in absorption (only 10%) when the drug was administrated in the salt form. In the case of elevated gastric pH, the differences in gastric pH due to omeprazole administration can explain the increase in variability.

3.3. The effect of food effect on inter-subject variability in absorption (intermediate 2 bioavailability compound)

For intermediate 2, the k_a value for the oral solution was relatively high compared to the PR formulation, indicating a slow release of the drug from the tablet matrix in the GI tract, and hence slow absorption. The variability in the rate of absorption was higher in the case of the solid dosage forms as compared to the solution, which might be attributed to the differences in the disintegration and dissolution of the drug resulting from the difference between individual GI physiology. With regards to absorption, solution absorption in the fasted state was estimated 60% and increased in the fed state (77%). Comparing the PR formulation and the solution in the fasted state, it can be seen that absorption increased by 10%. In addition, no food effect was observed for the PR formulation (71% vs. 68% under fast and fed states respectively).

The physiology of the gastrointestinal tract changes in the fed state, and may consequently affect drug absorption. The remarkable changes in the stomach under the fed state notably include a rise in gastric pH thanks to buffering and dilution effects, along with an increase in the gastric fluid volume and a decrease in gastric emptying time. In the small intestine, an increase in bile salt concentration, decrease in fluid volumes and in some cases inhibition of CYP enzymes and efflux transporters are expected (5). Since intermediate 2 is a free base, it would be expected to have high solubility in the gastric fluids, and its solubility should not decrease significantly in the administered clinical dose in the intestine. Another explanation is the drug degradation in low pH conditions which might explain the increased absorption under fed state. *In vitro* studies have shown to support this hypothesis (11). In the fed state, both the elevated gastric pH and the low retention time in the stomach might contribute to the drug stability, and

therefore more drug arriving to the small intestine that is available for absorption. In addition, it might be that an increase in bile salt concentration and gastric fluid volumes might have a positive food effect on the drug absorption under fed conditions. The food effect vanished in the case of the prolonged release formulation. Thanks to a slower dissolution in the stomach, less of the drug is deemed susceptible to degradation in the acidic conditions of the stomach, and more available to be absorbed in the small intestine.

High inter-variability (greater than 30%) can be attributed to the absorption process for all formulations. Although a positive food effect caused an increase in absorption in the fed state, formulating the drug as prolonged-release tablet did not reduced the variability in absorption in either the fasted or fed states. It might be that other physiological conditions (i.e. transit time) contributing to the inter-subject variability in absorption.

In this study, a model to estimate inter subject variability in absorption was developed by implementing the well stirred model using population pharmacokinetics. For formulation scientists, the input of formulation performance with regard of variability might increase the understanding of absorption mechanism and physiological factors affecting the drug absorption in particular for compounds classified as BCS II, to enable formulation optimisation to reduce the risk for high variability. This method to estimate variability in absorption can also be useful for clinical pharmacology scientist in planning and designing clinical trials in later stages of drug development to optimise sampling time and the number of subjects to enrol the study which will have great input for the clinical trial size.

4. Conclusions

Bioavailability is a commonly-used but complex and error-inherent means by which drug absorption is estimated in clinical trials and beyond drug development. Here, the well-stirred model was successfully implemented to delineate absorption from metabolism using a population pharmacokinetics approach. Our method maybe especially important for optimising formulation development for compounds with low and intermediate bioavailability. From our results, we have shown that absorption is solubility/dissolution-limited for intermediate 1 compound, likely attributable to significant drug precipitation in the small intestine. Due to the basic nature of the Intermediate 1 compound, we estimated low absorption on exposure to an elevated gastric pH, though there was evidence of considerable inter-subject variability. By comparison, food effects influencing absorption of the intermediate 2 compound disappeared when the drug was administered in a prolonged release formulation, indicating that in the absence of food, the oral solution is less stable on exposure to an acidic gastric pH. This investigation in early stages of drug development with the support of in vitro data, will contribute to our mechanistically understanding of the factors contributing drug absorption variability, assist in planning future clinical trials and power them accordingly. In addition, it can support label information (i.e. restriction for PPI's and food effect).

Estimations for drug absorption in this work included the fraction that escape gut wall metabolism. To our knowledge, there is no definite method to calculate f_g from plasma concentration vs. time data, though obtaining a separate estimation of f_g by developing a separate model remains highly desirable. Estimation of absorption from phase I/II clinical studies would otherwise enable better understanding of the factors contributing to low and erratic absorption, and therefore aid selection of the most appropriate formulation for further development. Moreover, a better understanding of drug absorption variability will enable better planning and execution of phase II and III clinical trials through aiding improved selection of sample size and dosage regimen, and so on, for the purpose of optimising the drug development process.

1. Chiou W. The Rate and Extent of Oral Bioavailability versus the Rate and Extent of Oral Absorption: Clarification and Recommendation of Terminology. Journal of Pharmacokinetics and Pharmacodynamics, 2001 Feb 1;28(1):3–6.

2. Huang W, Lee S, Yu L. Mechanistic Approaches to Predicting Oral Drug Absorption. AAPS J. 2009 Jun 1;11(2):217–24

3. Jamie M, Dickinson GL, Rostami- Hodjegan A. A Framework for Assessing Inter-individual Variability in Pharmacokinetics Using Virtual Human Populations and Integrating General Knowledge of Physical Chemistry, Biology, Anatomy, Physiology and Genetics: A Tale of "Bottom-Up" vs "Top-Down" Recognition of Covariates. Drug Metabolism and Pharmacokinetics. 2009;24(1):53–75.

4. Jamei M, Turner D, Yang J, Neuhoff S, Polak S, Rostami-Hodjegan A. Population-Based Mechanistic Prediction of Oral Drug Absorption. The AAPS Journal. 2009 Jun;11(2):225–37.

5. Varum F, Hatton G, Basit A. Food, physiology and drug delivery. International journal of pharmaceutics. 2013;457(2):446–60.

6. Pillai G.C., Mentré F., Steimer J.-L. (2005) Non-linear mixed effects modeling–from methodology and software development to driving implementation in drug development science. Journal of Pharmacokinetics and Pharmacodynamics 32:161-183.

7. Noda, T., Todani, T., Watanabe, Y., and Yamamato, S. (1997). Liver volume in children measured by computed tomography. Pediatr. Radiol. 27(3):250–252.

8. Holford NG. A Size Standard for Pharmacokinetics. Clin-Pharmacokinet. 1996 May 1;30(5):329–32.

9. Rowland M, Tozer T. Concepts and applications. Clinical pharmacokinetics. 1995;3.

10. Sigfridsson K, Lundqvist R, Ohlson K. Preformulation evaluation of AZD1305, an oxabispidine intended for oral and intravenous treatment. Drug development and industrial pharmacy. 2012;38(1):19–31.

11. Carlert S, Pålsson A, Hanisch G, von Corswant C, Nilsson C, Lindfors L, et al. Predicting Intestinal Precipitation—A Case Example for a Basic BCS Class II Drug. Pharm Res. 2010 Oct 1;27(10):2119–30.