British Journal of Applied Science & Technology
5(6): 613-620, 2015, Article no.BJAST.2015.059
ISSN: 2231-0843

SCIENCEDOMAIN *international*
www.sciencedomain.org

Investigation of Low Bioavailability Using Physiologically Based Pharmacokinetic Modeling: A Case Example (Lassbio-596)

Manuela de Lima Toccafondo Vieira[1,2], Lídia Moreira Lima[1], Eliezer J. Barreiro[1] and Carlos Alberto Tagliati[1,2*]

[1]National Institute of Science and Technology of Drugs and Medicines, Laboratory of Evaluation and Synthesis of Bioactive Substances (LASSBio®), Federal University Federal of Rio de Janeiro, CCS, Cidade Universitária, P.O. Box 68006, ZIP: 21941-971, Rio de Janeiro-RJ, Brazil.
[2]Departament of Clinical Analysis and Toxicological, Laboratory of Experimental Toxicology, School of Pharmacy, Federal University Federal of Minas Gerais, Av. Antônio Carlos, 6627, Pampulha, ZIP: 31270-901, Belo Horizonte-MG, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Author MDLTV designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors LML, EJB and CAT managed the analysis of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJAST/2015/13527
Editor(s):
(1) Maria Laura Mastellone, Environmental and Health Sciences, Second University of Naples, Italy.
(2) Ya-mei Gao, College of Life Science and Technology, Heilongjiang Bayi Agariculture Univ., Daqing, Heilongjiang, China.
Reviewers:
(1) Usman Sumo Friend Tambunan, Department of Chemistry, Faculty of Mathematics and Science, University of Indonesia, Indonesia.
(2) Allegaert Karel, Department of Development and Regeneration, KU Leuven, Belgium.
(3) Jeff Fisher, National Center for Toxicological Research, US FDA, 3900 NCTR Road, Jefferson, AR 72079, USA.
(4) Anonymous, University of Pisa, Italy.
(5) Muralidharan S, Chemistry, AIMST University Malaysia, Malaysia.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=763&id=5&aid=7076

Method Article

Received 21st August 2014
Accepted 10th November 2014
Published 24th November 2014

ABSTRACT

Aim: Investigate the possible mechanism (s) of the poor bioavailability of a lipophilic compound in rats using the physiologically based pharmacokinetic (PBPK) modeling approach.
Methodology: A rat PBPK model was constructed using data from intravenous administration, and verified by comparing predicted tissue concentrations (kidneys, liver and lungs) with experimental

**Corresponding author: E-mail: carlostagliati@ufmg.br;*

data from tissue distribution studies. Using parameter sensitivity analysis, the model was used to investigate the absorption characteristics of the compound and the probable causes of the low absorbed fraction.

Results: Sensitivity analysis of absorption parameters was performed to understand the absorption characteristic of the compound in regard to permeability, solubility and intra-gut degradation. Taking in consideration the latter factor, the oral pharmacokinetics of the tested compound was satisfactorily predicted in rats.

Conclusion: The PBPK simulation results suggest that chemical or/and bacterial degradation of the compound in the gastrointestinal tract may be a probable cause of the low bioavailability observed.

Keywords: Physiologically-based pharmacokinetic; pharmacokinetic; bioavailability; modeling.

NOMENCLATURE AND UNITS

LogP = partition coefficient; pKa = dissociation constant; B/P = blood-to-plasma partition ratio; fu,p = fraction unbound in plasma; Vss = volume of distribution at steady state; Kp = tissue-to-plasma partition coefficient; CLiv = intravenous clearance; CLr = renal clearance; Fa = fraction absorbed.

1. INTRODUCTION

Characterizing the role of the mechanisms that drive the oral bioavailability of a drug candidate is of particular interest in the early phases of drug development. For orally administered drugs, poor solubility, poor permeability, interaction with intestinal efflux transporters, extensive first-pass metabolism and/or gut degradation (chemical or bacterial) can be limiting factors for reaching desirable bioavailability. Understanding the limitations to absorption arising from these factors can be derived from available biopharmaceutical properties, in vitro data and preclinical species pharmacokinetics using pragmatic and mechanistic approaches, such as the physiologically based pharmacokinetic (PBPK) modeling.

Physiologically based pharmacokinetic modeling strategy has been increasingly used during drug discovery and development process [1]. Recent studies have demonstrated the value of PBPK in prospective predictions of absorption, distribution and clearance to aid on clinical candidate nomination [2-5].

PBPK modeling account for all relevant processes affecting oral drug absorption, including physiological factors, formulation and drug-specific characteristics, and consider the interplay of these processes in determining the rate and extent of absorption from the gastrointestinal tract [1].

This work presents a case example of PBPK modeling investigation of the probable mechanism(s) of the poor bioavailability of the compound LASSBio-596 in rats. The compound is not metabolized by CYP enzymes (in vitro study) and is not a P-gp substrate (in vivo study). The role of intrinsic intestinal permeability and gut degradation on LASSbio-596 bioavailability was assessed using sensitivity analysis of model parameters.

2. METHODOLOGY

2.1 Investigational Compound

The test compound LASSBio-596(2-{[4-(thiomorpholine-4-sulfonyl)phenyl]carbamoyl}benzoic acid, $C_{18}H_{18}N_2O_5S_2$, Fig. 1) is anantiasthmatic drug candidate at the preclinical stage of development [6].

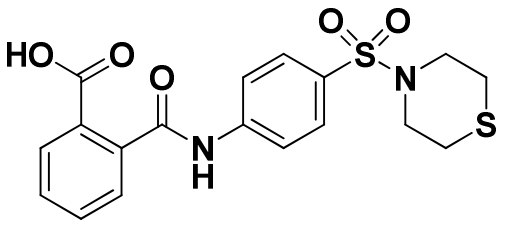

Fig. 1. Molecular structure of LASSBio-596

2.2 Development of LASSBio-596 PBPK Model

PBPK model of the compound Lassbio-596 was build using SimCYP® population-based simulator (Version 12 release 1, Sheffield, UK)

[7]. Model development and simulations were conducted in a virtual rat PBPK model with system dependent parameters built-in in the software. The rat PBPK model was composed of eleven compartments corresponding to the different body tissues/organs, which were defined by tissue volume, composition and blood flow rate specific for the species (Fig. 2).

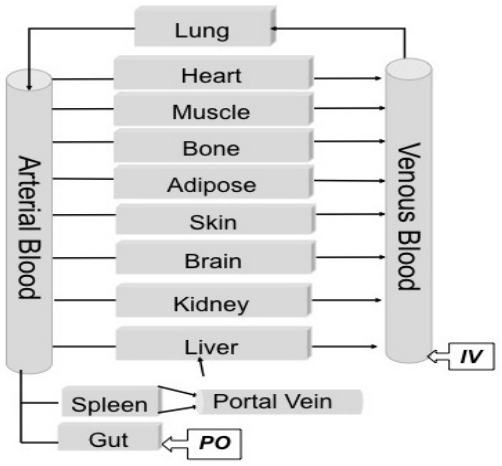

Fig. 2. Schematic representation of the full physiologically based pharmacokinetic (PBPK) model used

The first step of model development included the use of in silico-predicted parameters and in vitro data to determine the compound's ADME parameters. The drug-dependent parameters of the compound LASSBio-596 are summarized in (Table 1). The physicochemical properties, partition coefficient (LogP), number of hydrogen bound donors (HBD) and polar surface area (PSA), were obtained from Chemspider (http://www.chemspider.com; Royal Society of Chemistry, Cambridge, United Kingdom) [8]. The compound's fraction unbound in plasma was experimentally determined by other group [6]. Since the blood-to-plasma ratio (B:P) was not available, it was assumed to be 0.57 (no hematocrity affinity) as suggested for acidic compounds [9]. The second step of model building included selection of the appropriate model structure for the prediction of distribution and clearance based on comparison with observed data after intravenous administration. The distribution parameters, volume of distribution at steady-state (Vss) and tissue-to-plasma partition coefficients for each tissue compartment, were predicted in silico using the algorithm published by Poulin and Thiel [10], based on the compound's physicochemical properties. The full PBPK model with perfusion rate limited for each tissue was assumed.

Data from *in vitro* metabolic studies demonstrated that LASSBio-596 was not metabolized by plasmatic esterase of rats, and was stable at rat liver microsomal fraction (unpublished data). Therefore, it is suggested that LASSBio-596 is not metabolized by rat cytochrome P450 (CYP450) enzymes, flavin monooxygenase, and carboxyestherases. Based on these *in vitro* results, the hepatic clearance was considered to be negligible and the PBPK model was build assuming that the compound is exclusively renally eliminated. To establish the compound total and renal clearance, the observed intravenous clearance after single 10 mg/kg bolus dose, 0.3 L/h/kg [4], was optimized to 1.2 L/h/kg to adequately recover the observed mean data of the compound's elimination profile. This optimization was deemed necessary for model building, and the impact of this assumption on the compound's model was assessed in the verification step of model development.

2.3 Verification of LASSBio-596 PBPK Model

Simulation of a scenario and data not used for model building was done to verify the initial PBPK model and assumptions made. To that end, LASSBio-596 PBPK model was evaluated by comparing the simulated compound concentration in the kidneys, liver and lungs tissues against the observed values obtained experimentally from a rat tissue distribution study.

2.4 Sensitivity Analysis of the Drug Parameters Related to the Absorption

The absorption kinetics of the tested compound was established using the Advance Dissolution Absorption and Metabolism (ADAM) model [11]. The parameters related to intestinal metabolism were the unbound fraction within the enterocyte (fugut, assumed equal to 1) and the nominal flow from gut model (Qgut, value predicted within the simulator) [12]. The absorption parameters fa and ka were predicted based on the molecule polar-surface area (PSA). The automatic sensitivity analysis (ASA) feature of the software was used to verify the influence of PSA values, and the gastric and intestinal degradation kinetics on the prediction of fa and ka.

Table 1. Drug-dependent parameter table for Lassbio-596

Parameter	Value	Methods/references
MW (g/mol)	406.48	Predicted by chemspider
LogP	2.14	Predicted by chemspider
Acidic pka	2.74	Calculated using MoKa software 1.1.1
B/P	0.57	Assumed no hematocrit affinity for acids [9]
fu	0.87	Determined by ultrafiltration [6]
$fu_{,gut}$	1	Assumed
fa	0.77	Predicted by SimCYP based on PSA
PSA ($Å^2$)	137.46	Predicted by chemspider[a]
HBD	2	Predicted by chemspider[a]
Aqueous solubility (mg/ml)	0.0262	(unpublished data)
Predicted V_{ss}(L/kg)	2.34	Predicted by method 1 [10]
Kps lung	3.028	Predicted by method 1 [10]
Kps kidney	2.392	Predicted by method 1 [10]
CLiv (mL/min)	5	Optimized based on initial observed value [6]
CL_r(mL/min)	5	Assumed based on lack of liver microsomal and plasma enzymes metabolism demonstrated in vitro (unpublished data)

LogP, partition coefficient; pKa, dissociation constant; B/P, blood-to-plasma partition ratio; fu,p, fraction unbound in plasma; Vss, volume of distribution at steady state; Kp, tissue-to-plasma partition coefficient; CLiv, intravenous clearance; CLr, renal clearance; fa, fraction absorbed, [a]ChemSpider (http://www.chemspider.com; Royal Society of Chemistry, Cambridge, United Kingdom)

Sensitivity analysis of model parameters was performed on a range of PSA values (13.75 to 300 Å2) to evaluate their impact on the compound's absorption characteristics. ASA was also done with a range of values of the degradation rate constants in rat stomach and intestine (kdeg 0 to 25 h-1) fluids to evaluate the role of intra-gut degradation on compound's absorption. The results of each ASA were plotted against the observed mean plasma concentration-time profile after oral administration to test their adequacy. The best-suited values were further simulated to obtain the oral PK profile and parameters.

3. RESULTS AND DISCUSSION

3.1 PBPK Model Development and Verification

The LASSBio-596 PBPK model was built using the rat physiological model within the software. Assuming a diffusion-limited distribution model, renal clearance as the major elimination pathway, and the optimized CLiv, the model reasonably predicted the plasma concentration–time profile compared with the observed intravenous administration data (Fig. 3).

The PBPK modeling performance was verified by comparing predicted compound concentrations in key tissues- kidneys, liver and lungs- against the experimentally determined values [6] (Fig. 4). After i.v. administration, LassBio-596 exposure in the kidneys over time (18 h) is adequate predicted by the PBPK approach (Fig. 4a). The compound's lung concentration-time profile is fairly recovered by the simulation as well (Fig. 4b). While the predicted concentration-time profile of LassBio-596 in the liver appears under-predicted (Fig. 4c), the experimental data may be over-predicting the actual liver exposure. The researchers suggested that the liver homogenate may present an excessive higher blood contamination due to the extensive organ vascularization. This practical caveat may contribute to the higher concentration determined in liver and relative tissue penetration [6]. Overall, the reasonably prediction of tissue exposure suggested that the mechanism and assumptions incorporated in the compounds PPBPK model are fairly adequate to allow further analysis. Following the model for intravenous dosing, the PBPK model for oral dosing was built by addition of compound-specific absorption parameters (Table 1).

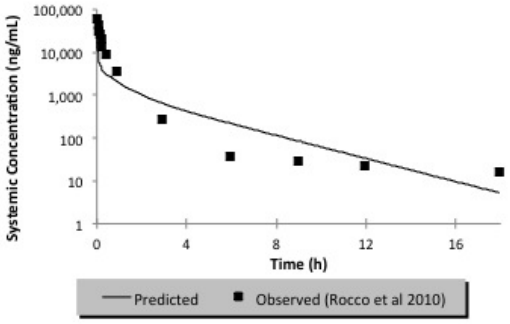

Fig. 3. PBPK predicted and observed systemic concentration–time profile of LASSBio-596 after intravenous administration (10 mg/kg iv bolus dose) in rats

3.2 Investigation of Possible Mechanism(s) of Low Bioavailability

In experimental rats, the oral dosing of an aqueous solution of the tested compound, at a single dose of 10 mg/kg, resulted in a very low bioavailability (F=3.6%).

Several mechanisms are associated with a compound's poor absorptive profile: Efflux from apical intestinal cells via the transporter P-gp [13], extensive first-pass metabolism [14], substrate intrinsic low intestinal permeability [15], and intra-gut degradation (chemical or bacterial) [16,17].

The role of the intestinal efflux transporter P-gp on the poor absorption of tested compound, LASSBio-596, was previously investigated in vivo [6]. Rats were treated with the P-gp inhibitor verapamil (40 mg/kg po) one hour before LASSBio-596 administration (10 mg/kg po). The absolute bioavailability determined in verapamil-treated animals, F=5%, was not significantly different from the control group (F= 3.6%). This finding suggests negligible contribution of the transporter P-gp on the absorption of LASSBio-596 [6].

In vitro metabolic studies demonstrated that the compound LASSBio-596 was not metabolized by plasmatic esterase of rats, and was stable to rat liver microsomal fraction (unpublished data). Therefore, LASSBio-596 is not metabolized by CYP450 enzymes, flavin monooxygenase, and carboxyesterase. The role of phase II enzymes, such as UDP-glucuronosyltransferases (UGTs), sulfotransferases (PSTs), methyl transferases, acetyl transferases and glutathione S-transferases (GSTs) in the metabolism of the tested compound was not evaluated. Since UGTs, PSTs and GSTs are also present in the intestinal wall, a simulation was performed assuming an extreme scenario of total contribution of hepatic and intestinal metabolism to the total clearance. The predicted effect of a hypothetical liver and intestinal first-pass metabolism did not recover the absorption properties of the compound (predicted fg=1, fg= 0.55, fa (fraction absorbed)=0.98 and F= 54%) and the observed PK profile (Fig. 5a). This finding confirmed that negligible contribution of the hepatic elimination pathway.

Since no in vitro study was performed to determine LASSBio-596 intestinal permeability, the compound's permeability was still predicted based on its physicochemical properties. The value of the molecule polar surface area was used to predict the absorption parameters, fa and ka (absorption rate constant). LASSBio-596 PSA value of 137 (together with HBD of 2) did not recover the observed low bioavailability (observed versus predicted F= 3.6% versus 97%, respectively), with the compound's exposure significantly over-predicted (Fig. 5b). PSA value is not considered the most appropriate surrogate to predict a substrate intrinsic permeability. Thus, sensitivity analysis was performed on a range of PSA values (13.75-300 Å2) to evaluate the impact on the absorption parameters. Results showed an inversed relationship between a PSA value and the fraction absorbed and AUC. The use of the highest PSA value possible, 300, was used to simulate LASSBio-596 plasma concentration-time after single oral 10mg/kg dose. As result of a hypothetical lower permeability, a poor fraction absorbed (0.41) and lower absorption rate still did not recover the observed PK profile (Fig. 5c), especially in regard to Tmax (observed versus predicted Tmax = 0.6 h versus 1.55 h, respectively) and bioavailability (observed versus predicted F = 3.6% versus 40%, respectively).

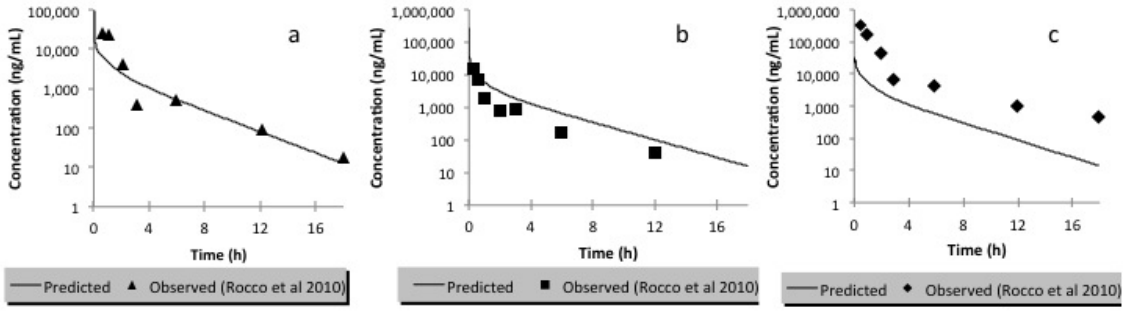

Fig. 4. PBPK predicted and observed tissue concentrations ((a) kidneys, (b) lung, and (c) liver) of LASSbio-596 over time after intravenous administration (10 mg/kg iv bolus dose) in rats

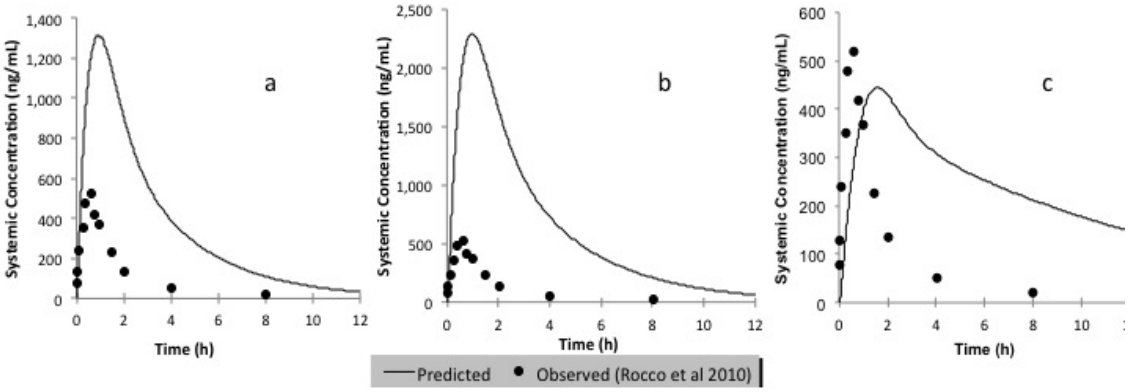

Fig. 5. PBPK predicted and observed systemic concentrations of LASSbio-596 over time after oral administration (10 mg/kg single dose) in rats. Simulations were performed to evaluate (a) hypothetical liver and intestinal first-pass metabolism (b) predicted permeability based on PSA value (c) hypothetical lower permeability

Finally, the possible role of chemical [16] or enzymatic (microbial) [17] degradation of a compound within the gut lumen was investigated. Sensitivity analysis was performed on a range of values of stomach and intestine degradation rate constants (k_{deg} 0 to 25 h^{-1}) to evaluate the role of intra-gut degradation on LASSBio-596 absorption parameters. The Cmax. AUC, Tmax and fa were sensitive to kdeg. Based on the results of sensitivity analysis, values of stomach k_{deg} of 10 h^{-1} and intestinal kdeg of 1.5 h^{-1} were used to simulate LASSBio-596 plasma concentration after single oral 10mg/kg dose. The assumption of an intra-gut degradation adequately recovered the c-time profile after oral dose (Fig. 6). Table 2 lists the comparison of the observed and PBPK predicted PK parameters, with gastrointestinal degradation incorporated in the model. There was an accurate prediction of the compound exposure after oral dosing in rats- the predicted F closely approximate to the observed value (observed versus predicted F = 0.04 versus 0.13, respectively) and the AUC was correctly forecast by the simulations (observed versus predicted AUC= 1.4 x 10^3 versus 1.09 x 10^3 ng/ml.h, respectively).The results let to the conclusion that intra-gut degradation may be the probable mechanism responsible for the poor bioavailability of LASSBio-596 in rats.

3.3 Limitations of the Current Study

Though the current study demonstrated the utility of PBPK modeling in predicting a probable mechanism of the low bioavailability of the compound LASSBio-596, some limitations should be noted. First, the effect of the dosing vehicle (0.9% NaCl solution containing 3% (v/v) polysorbate 80) on the absorption of the compound was not evaluated. Second, faecal excretion of unabsorbed compound is also a possibility for the poor bioavailability. This

hypothesis can be verified in a mass balance study. Third, the contribution of phase II enzymes in the metabolism of LASSBio-596 was not investigated. Further research is needed to address the role of these factors on the compound's absorption and clearance properties.

Table 2. PBPK predicted and observed pharmacokinetic parameters of LASSbio-596 after oral administration (10 mg/kg single dose) in rats. Simulation was performed incorporating the constants of intra-gut degradation

PK parameters	Observed	Predicted
Tmax (h)	0.6	0.4
Cmax (ng/ml)	493	526
AUC (ng/ml.h)	1.4×10^3	1.09×10^3
F	0.04	0.13
t ½ terminal (h)	2.0	1.35

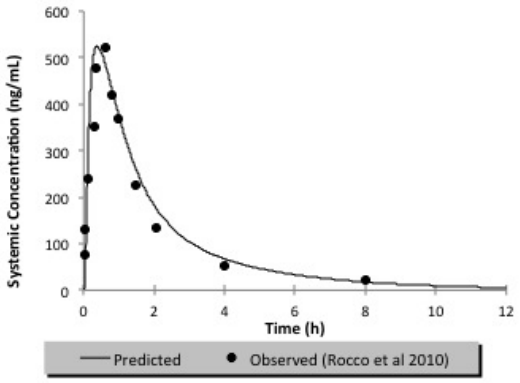

Fig. 6. PBPK predicted and observed systemic concentrations of LASSbio-596 over time after oral administration (10mg/kg single dose) in rats. Simulation was performed incorporating the constants of intra-gut degradation

4. CONCLUSION

This study demonstrated the practical use of PBPK modeling to investigate the probable mechanism(s) of the observed poor bioavailability of an investigational compound in rats. A good understanding of the absorption properties of a drug candidate and their role in the oral bioavailability may aid on lead optimization strategies and decrease compound attrition during drug development. Mechanistic approaches such as PBPK modeling and simulation can be a very effective tool to generate hypotheses around the limiting factors affecting a desirable drug PK profile. By providing the most information of data available at early stages of drug development, such as in vitro and preclinical data, PBPK approach may also aid on reduction of costs and time on development.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kostewicz E, Aarons I, Bergstrand M, Bolger M, Galetin A, Hatley O, et al. PBPK models for the prediction of in vivo performance of oral dosage forms. European Journal of Pharmaceutical Sciences, Philadelphia; 2013. [epub ahead of print] DOI: pii: s0928-0987(13)00362-x.10.1016/j.ejps.2013.09.008.
2. Otsuka K, Shono Y, Dressman J. Coupling biorelevant dissolution methods with physiologically based pharmacokinetic modelling to forecast *In vivo* performance of solid oral dosage forms. Journal of Pharmacy and Pharmacology. 2013;65(7):937-952.
3. Sinha VK, Snoeys J, Osselaer NV, Peer AV, Mackie C, Heald D. From preclinical to human--prediction of oral absorption and drug-drug interaction potential using physiologically based pharmacokinetic (PBPK) modeling approach in an industrial setting: A workflow by using case example. Biopharmarceutical Drug Disposition. 2012;33(2):111-121.
4. Chen Y, Jin JY, Mukadam S, Malhi V, Kenny JR. Application of IVIVE and PBPK modeling in prospective prediction of clinical pharmacokinetics: Strategy and approach during the drug discovery phase with four case studies. Biopharmarceutical Drug Disposition. 2012;33(2):85-98.
5. Hu ZY, Lu J, Zhao Y. A physiologically based pharmacokinetic model of alvespimycin in mice and extrapolation to rats and humans. British Journal of Pharmacology. 2014;171(11)2778-2789.
6. Rocco P, Xisto D, Silva J, Diniz M, Almeida R, Luciano M, et al. Lassbio-596: da descobertaaosensaiospré-clínicos." Lassbio-596: Fromdiscovery to pre-

clinicalstudies". RevistaVirtual de Quimica. 2010;2(1):10-27.

7. Jamei M, Dickinson G, 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 Metabolismand Pharmacokinetic. 2009;24(1):53-75.
8. Chemspider Home. Royal Society of Chemistry, Cambridge, United Kingdom. Acessed December 1st, 2013. Available: http://www.chemspider.com
9. Riley R, Mcginnity D, Austin R. A unified model for predicting human hepatic, metabolic clearance from in vitro intrinsic clearance data in hepatocytes and microsomes. Drug Metabolism and Disposition. 2005;33(9):1304-1311.
10. Poulin P, Theil F. A priori prediction of tissue: Plasma partition coefficients of drugs to facilitate the use of physiologically-based pharmacokinetic models in drug discovery. Journal of Pharmaceutical Sciences. 2000;89(1):16-35.
11. Jamei M, Turner D, Yang J, Neuhoff S, Polak S, Rostami-hodjegan A, Tucker G. Population-based mechanistic prediction of oral drug absorption. The American Association of Pharmaceutical Scientists Journal. 2009;11(2):225-237.
12. Yang J, Jamei M, Yeo KR, Tucker G, Rostami-Hodjegan A. Prediction of intestinal first-pass drug metabolism. Current Drug Metabolism. 2007;8(7):676-684.
13. Murakami T, Takano M. Intestinal efflux transporters and drug absorption. Expert Opinion on Drug Metabolism & Toxicology. 2008;4(7):923-939.
14. Shen D, Kunze K, Thummel K. Enzyme-catalyzed processes of first-pass hepatic and intestinal drug extraction. Advanced Drug Delivery Reviews. 1997;27(2-3):99-127.
15. Valko K, Butler J, Eddershaw P. Predictive approaches to increase absorption of compounds during lead optimisation. Expert Opinion of Drug Discovery. 2013;8(10):1225-1238.
16. Di l, Kerns E, Chen H, Petusky S. Development and application of an automated solution stability assay for drug discovery. Journal of Biomolecular Screening. 2006;11(1):40-47.
17. Sousa T, Paterson R, Moore V, Carlsson A, Abrahamsson B, Basit A. The gastrointestinal microbiota as a site for the biotransformation of drugs. International Journal of Pharmaceutics. 2008;363(1-2):1-25.

© 2015 Vieira et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=763&id=5&aid=7076