

Application of *in Silico* Tools in Clinical Practice using Ketoconazole as a Model Drug

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ABSTRACT - Hypochlorhydria is a condition where the production of hydrochloric acid in the stomach is decreased. As a result, the intragastric pH is elevated. This condition can be due to a series of causes, such as disease (gastric mucosal infection caused by *Helicobacter pylori* and is prominent in AIDS patients), ethnicity, age and also the use of antisecretory agents. This may significantly impact the absorption of other drugs that have pH-dependent solubility, such as ketoconazole, a weak base. Within this context, the purpose of this study was to demonstrate how GastroPlus™ – a physiological based software program- can be used to predict clinical pharmacokinetics of ketoconazole in a normal physiological state vs. elevated gastric pH. A simple physiologically based pharmacokinetic model was built and validated to explore the impact that different physiologic conditions in the stomach (hypochlorhydria, drug administered with water and Coca Cola®) had on ketoconazole's bioavailability. The developed model was able to accurately predict the impact of increased pH and beverage co-administration on dissolution and absorption of the drug, and confirmed that complete gastric dissolution is essential. Particle size only mattered in hypochlorhydric conditions due to the incomplete gastric dissolution, as its absorption would depend on intestinal dissolution, which corroborates with previous studies. Therefore, *in silico* approaches are a potential tool to assess a pharmaceutical product's performance and efficacy under different physiological and pathophysiological states supporting the assessment of different dosing strategies in clinical practice.

INTRODUCTION

Hypochlorhydria is a physiological state in which the hydrochloric acid production in the stomach is low, causing an increase in the intragastric pH. Disease state (gastric mucosal infection caused by *Helicobacter pylori* and AIDS patients), ethnicity, age and administration of antisecretory agents, such as omeprazole, a Proton Pump Inhibitor (PPI), may induce hypochlorhydria¹⁻³. Changes in physiological properties such as stomach pH can impact the *in vivo* drug product performance, especially from dosage forms with API controlled dissolution^{4,5}. Thus, antisecretory agents can affect the absorption of orally co-administered drugs, given that gastric acidity plays a major role in the process of dissolution sequentially followed by absorption of various drugs^{3,6}. This is the case for drugs that are primarily weak bases.

A clinical example of this is the administration of ketoconazole in PPI-induced hypochlorhydria states. Several studies^{3,7-9} have reported its reduced absorption under such conditions. Ketoconazole is an antifungal agent with a broad-spectrum activity against various fungal infections¹⁰. It is used to treat both mucocutaneous and systemic opportunistic fungal infections that

commonly occur in immunocompromised patients^{7,8,11}. It is a chiral imidazole piperazine compound, which is a weak dibasic compound⁷, and within the Biopharmaceutics Classification System (BCS)¹², it is a low soluble and highly permeable drug (class II)¹³. Since ketoconazole solubility is pH dependent, its absorption after oral administration is variable, achieving the highest plasma concentrations at low gastric pH^{8,14}. Due to its high lipophilicity, ketoconazole is readily absorbed after conversion to the water-soluble salt by gastric acid¹⁵⁻¹⁷.

Under this scenario of different physiological conditions, physiologically based pharmacokinetic (PBPK) computer models are useful tools to help predict the plasma concentration–time profiles of a given drug. GastroPlus™ (Simulations Plus Inc., Lancaster, CA, USA) is an example of commercially available software that includes PBPK models¹⁸. Using pre-determined *in vitro* parameters and physiology, PBPK modeling can

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predict *in vivo* data, improving the therapeutic outcomes by designing different disposition profiles^{2,19,20}. Much attention has been drawn to the use of such models for drug development and formulation development process^{21–25}, nevertheless there is a great opportunity in using simulations to investigate different clinical approaches by clinical practitioners.

In order to predict drug absorption from the gastrointestinal (GI) tract, GastroPlus™ includes the Advanced Compartmental Absorption and Transit (ACAT) model²⁶, which is a refinement of the Compartmental Absorption and Transit (CAT) model^{27,28}. This model takes into consideration factors that impact drug bioavailability and absorption, such as physicochemical attributes of the compound (e.g. solubility), physiological properties of the GI tract (e.g. pH) and formulation characteristics (e.g. particle size)^{20,26,28}.

The purpose of this study was to demonstrate the use of *in silico* studies to support changes in clinical practice with a mechanistic approach in view.

MATERIALS AND METHODS

MATERIALS

Ketoconazole USP grade was purchased from Medisca, (Saint-Laurent, QC; LOT: 613650/D). Coca Cola® and Orange juice (Minute Maid®) were bought at a local store in Edmonton, Canada. Water for the high-performance liquid chromatography (HPLC) assay was purified by Elgastat Maxima UF and an Elgastat Option 3B water purifier by ELGA Laboratories Ltd. (Mississauga, ON, Canada) and then filtered using 0.45 µm pore size filter. Methanol and acetonitrile used were HPLC grade and were purchased from Fisher Scientific (Fair Lawn, NJ, USA).

METHODS

Systematic search for clinical studies reporting the Ketoconazole malabsorption due to increased gastric pH

Databases such as Medline, Scopus, Web of Science, Google Scholar, Scencedirect and Scifinder were systematically searched to identify relevant studies using key-words alone and in combination with each other such as: pH, absorption, solubility, antisecretory therapy, ketoconazole, omeprazole, ranitidine, cimetidine, gastric pH and co-administration. Clinical studies performed on adult humans reporting ketoconazole malabsorption due to gastric acid secretion

inhibition by the use of PPIs and/or Histamine 2-receptor antagonists were selected.

Chemical structure analysis

The database chemicalize (<http://www.chemicalize.org/>)²⁹ was used to analyze ketoconazole chemical structure and its ionization characteristics throughout the pH range 0- 14.

Computer simulations using GastroPlus™

GastroPlus™ version 9.0 (Simulations Plus Inc., Lancaster, CA, USA) is a computer program that allows the prediction of drug absorption from oral administration of dosage forms when physicochemical and biopharmaceutical properties of drugs are available^{18–20,30–33}. The program is composed of different input tabs, such as Compound Tab, Gut Physiology and Pharmacokinetics. The other two tabs (Simulation and Graph) display the results for the simulations performed. The parameters and modules used in each tab for the different sets of simulation are described in detail.

Compound Tab

In the Compound Tab, ketoconazole physicochemical properties were input as shown in Table 1. These parameters comprise but are not limited to: dose, dosage form, solubility, permeability, molecular weight, particle density, particle size and pKa.

Even though being a weak base, the literature reports that ketoconazole is a slow precipitating drug^{34–36}. Hence, the default value of precipitation time was found to be adequate for this simulation setting.

Pharmacokinetics Tab

Since no intravenous human study has been reported to date,³⁶ published data with the administration of ketoconazole 200 mg oral suspension⁴² was used to set the human pharmacokinetics of ketoconazole using the physiologically based pharmacokinetics (PBPK) model in GastroPlus™. In the Compound Tab the Dosage Form selected was Immediate Release (IR) Suspension.

PBPKPlus™ (Simulations Plus Inc., Lancaster, CA, USA) is an additional module in GastroPlus™ that enables the prediction of the drug's distribution and clearance for all tissue compartments such as gut, lung, adipose tissue, muscle, liver, spleen, heart, brain, kidney, skin, and rest of the body that are interconnected by the systemic vasculature circulation^{18,36,43}.

Table 1. Drug properties used as input data in Compound Tab in GastroPlus™

Compound Tab Inputs	Value	Reference
Molecular weight (g/mol)	531.44	^a ADMET Predictor™
Permeability (10 ⁻⁴ cm/s)	3.7	37
pKa (Dibasic compound)		7,38,39
pKa1	2.9	
pKa2	6.5	
LogP	3.74	*ADMET Predictor™
pH for reference solubility	4.4	40
Solubility (mg/ml)	0.5	
Initial dose (mg)	200	41
Dose volume (ml)	250	GastroPlus™ default value
Drug particle density (g/ml)	1.2	GastroPlus™ default value
Mean precipitation time (s)	900	GastroPlus™ default value
Diffusion coefficient (cm ² /s x 10 ⁵)	0.56	*ADMET Predictor™

^aADMET Predictor™ module in GastroPlus™ was used to predict drug properties from its molecular structure.

When using PBPK simulations for small molecules a partition coefficient between tissue and plasma has to be set. This tissue/plasma partition coefficient (K_p) is a means to measure the amount of drug in the tissue and it can be estimated from physicochemical properties such as logP, pKa, unbound fraction of drug in plasma and blood/plasma concentration ratio⁴³. A modified Rodgers and Rowland predictive method present in GastroPlus™ was selected to calculate the K_p s.

Perfusion-limited kinetics with no concentration gradient in the tissue was used, given that ketoconazole is a highly lipophilic and highly permeable molecule and is classified as a BCS class II compound^{13,36}. Therefore it is reasonable to assume that the amount of drug that partitions into the tissue is limited by the blood flow rate through the tissue (perfusion rate) rather than permeability and surface area and partitioning

is instantaneous⁴³. The scheme in Figure 1 helps to illustrate this process.

Ketoconazole's major route of excretion is through the bile into the GI tract with more than 50% being excreted in the feces^{11,44,45}. Hence the hepatic clearance (CL_{hep}) was set equal to oral clearance (CL_{po}) of 12.5 L/h for a 200 mg dose^{36,42}. The default physiology (Human Physiological Fasted) was used.

Physiology Tab

Three sets of simulations were performed, and the predicted absorption was compared to the experimental data for the different physiology conditions in the stomach. The first set of simulations aimed to build a PBPK model using published data of ketoconazole 200 mg suspension administration⁴². Once the model was built, the same pharmacokinetics parameters obtained were used to run further simulations.

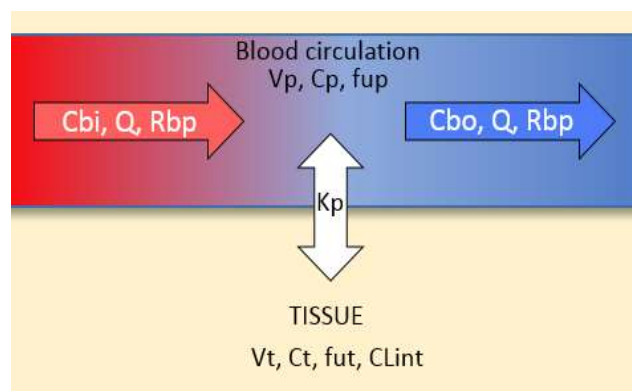


Figure 1. Scheme of perfusion-limited tissue. Vt: tissue volume; Ct: tissue concentration, fut: fraction unbound in tissue, CL_{int}: tissue intrinsic clearance; Vp: plasma volume; Cp: plasma concentration; fup: fraction unbound in plasma; C_{bi}: blood concentration in (arterial) tissue; Rbp: blood/plasma concentration ratio; Q: tissue blood flow; C_{bo}: blood concentration out (venous) of tissue; K_p: tissue/plasma partition coefficient. (Image adapted from GastroPlus™ Manual, 2015).

The other two sets of simulations comprised of ketoconazole 200 mg in an immediate release (IR) tablet dosage form for both normal and increased stomach pH. For those two sets of simulation, the dosage form in the compound tab was set to “IR: Tablet”.

These three sets of simulations: PBPK model; human fasted – no hypochlorhydria physiology and human fasted - hypochlorhydria physiology are described below and presented in Table 2.

Physiology Based Pharmacokinetics (PBPK) model

As mentioned above, a clinical study using ketoconazole 200mg oral suspension⁴² was selected to build the PBPK model. Since this study was conducted in humans in a fasted state, the default values in GastroPlus™ for human fasted physiology were used, as shown in Table 2. The default absorption model (Opt logD Model SA/V 6.1 model) and perfusion limited kinetics were used.

Human fasted - Normal intragastric pH - No hypochlorhydria

Experimental data were taken from a published study using ketoconazole tablets 200 mg (Nizoral; Janssen Pharmaceutica Inc., Mississauga, Ontario, Canada)⁷. A parameter sensitivity analysis (PSA) was performed on gastric transit time since the dosage form was changed from suspension to tablet, and it was set to one hour. It is reasonable to use a longer gastric transit time given that a solid oral dosage form takes longer to be emptied out of

the stomach when compared to a suspension.

Human fasted - Co-administration with PPI – Hypochlorhydria

Data from a study performed in subjects receiving 60 mg of omeprazole (Losec; Astra Pharma Inc.) on the night prior to receiving ketoconazole 200 mg was used⁷. The experimental data were compared with the simulations performed at increased intragastric pH. Stomach pH was set to 6.9, as reported in the study (Table 2). Besides that, gastric retention time was set to one hour to account for the difference between tablet and suspension dosage form and their gastric emptying rate.

The absorption scale factor (ASF) of the ACAT model was optimized. ASF is used to scale the effective permeability to account for various absorption-rate-determining effects, such as pH effects^{20,43}.

The improvement of ketoconazole’s absorption with administration of drug with an acidic beverage (Coca Cola®) in the presence of drug-induced hypochlorhydria was also assessed. The data was taken from the aforementioned study⁷. In this case, the stomach pH was decreased from 6.9 to 5.5.

Simulation and Graph Tab

The temporal length for the simulations was adjusted for each study according to the corresponding available data. Once the simulation for the plasma concentration curve was complete, the predicted curve was displayed in the Graph tab,

Table 2. ACAT parameters used in Physiology Tab for human fasted – PBPK model, normal and hypochlorhydria physiologies.

Physiology	Human fasted - PBPK model		Human fasted – Normal - No hypochlorhydria		Human fasted - hypochlorhydria	
	pH	Transit time (h)	pH	Transit time (h)	pH	Transit time (h)
Stomach	1.30	0.25	1.3	1	6.90	1
Duodenum	6.0	0.26	6.0	0.26	6.0	0.26
Jejunum proximal	6.2	0.93	6.2	0.93	6.2	0.93
Jejunum distal	6.4	0.74	6.4	0.74	6.4	0.74
Ileum proximal	6.6	0.58	6.6	0.58	6.6	0.58
Ileum medial	6.9	0.42	6.9	0.42	6.9	0.42
Ileum terminal	7.4	0.29	7.4	0.29	7.4	0.29
Caecum	6.4	4.31	6.4	4.31	6.4	4.31
Asc Colon ^a	6.8	12.93	6.8	12.93	6.8	12.93

^aAsc Colon = ascending colon

which automatically generates the regression coefficient (R^2) between predicted and observed data, along with other statistical parameters: sum of squared errors (SSE), root mean squared error (RMSE) and mean absolute error (MAE).

Since ketoconazole is a BCS class II drug¹³ the influence of particle size on drug absorption was investigated by running a PSA. The particle size range analyzed was 2-250 μm . After running a PSA, the Graph tab displayed the results delineating the particle size statistics effect on ketoconazole's pharmacokinetics.

DATA ANALYSIS

The data was analyzed through statistical parameters such as the sum of squared errors (SSE), root mean squared error (RMSE) and mean absolute error (MAE). The SSE measures the discrepancy between the model and data. Therefore, the smaller the value, the better the fit is. The differences between the predicted values and observed values is given by the RMSE. It combines the magnitude of the errors for the various time points into a single measure. It is a non-negative value and the closer to zero the better the fit. MAE, as the name indicates, is the average of all absolute errors.

Besides that, the pharmacokinetic parameters AUC, T_{max} and C_{max} were assessed in terms of the fold error between the observed and the predicted values, according to equation (1). As it is widely applied within pharmaceutical industries, a two-fold error was considered to be an acceptable prediction⁴⁶⁻⁵².

$$\text{Fold Error} = \frac{\text{Predicted value}}{\text{Observed value}} \quad (1)$$

Solubility test

The solubility of ketoconazole was determined using the equilibrium solubility test (Shake flask method). Four different media were tested in triplicate: Simulated gastric fluid (SGF) pH 1.2, SGF pH 5.0, Coca-Cola® and Orange Juice (Minute Maid®), the latter two were utilized directly from the commercial products. Each medium (5ml) was saturated with ketoconazole pure drug powder and there was no mixture of media. The flasks were shaken for 24 hours at room temperature to assure equilibrium. At equilibrium, the pH in each flask was measured.

Samples from each medium (SGF pH1.2; SGF

pH 5; Orange juice and Coca-Cola) were centrifuged for 10 min at 11900xg and the supernatant was diluted. The supernatant of Orange juice and Coca-Cola samples was diluted with methanol and the supernatant from SGF pH 1.2 and pH 5 was diluted with acetonitrile. The diluted supernatant from all samples was then centrifuged again for 10 min at 4400xg. The resulting supernatant was used in the HPLC assay. The mobile phase for the HPLC assay was composed of methanol, water and diethylamine 74:26:0.1 (v/v/v) and a Lichrospher® 60 RP Select B column (5 μm , 12.5×4 mm) column was used⁵³.

RESULTS

Chemical structure analysis

The graph below (Figure 2), retrieved from the Chemicalize database (<http://www.chemicalize.org/>), shows the result for the ionization microspecies distribution throughout the pH range 0 to 14. The green line represents the microspecies with both basic groups - imidazole and piperazine - protonated, the blue line is the neutral form and the orange line is the microspecies with only the imidazole group protonated (Figure 2).

PBPK model

Using the stated conditions, GastroPlus™ was able to closely predict the observed data for ketoconazole 200 mg suspension administration ($R^2 = 0.95$, SSE= 1.92, RMSE= 0.438, MAE= 0.33), as shown in Figure 3A. Also, the values of Area Under the Curve (AUC), C_{max} and T_{max} for the predicted data showed a good match to the observed parameters (Table 3). All the simulated parameters were within 2-fold error of the observed pharmacokinetic parameters.

Human fasted - Normal intragastric pH - No hypochlorhydria

The simulated plasma concentration curve for ketoconazole in normal gastric pH state resulted in a very good prediction for the observed plasmatic concentration curve ($R^2 = 0.913$, SSE= 2.776; RMSE= 0.502; MAE= 0.394) (Figure 3B). The pharmacokinetics parameters $AUC_{0-\infty}$, C_{max} and T_{max} and are summarized in Table 4, showing a well-defined match between the predicted and observed data. All the simulated parameters were within 2-fold error of the observed pharmacokinetic parameters.

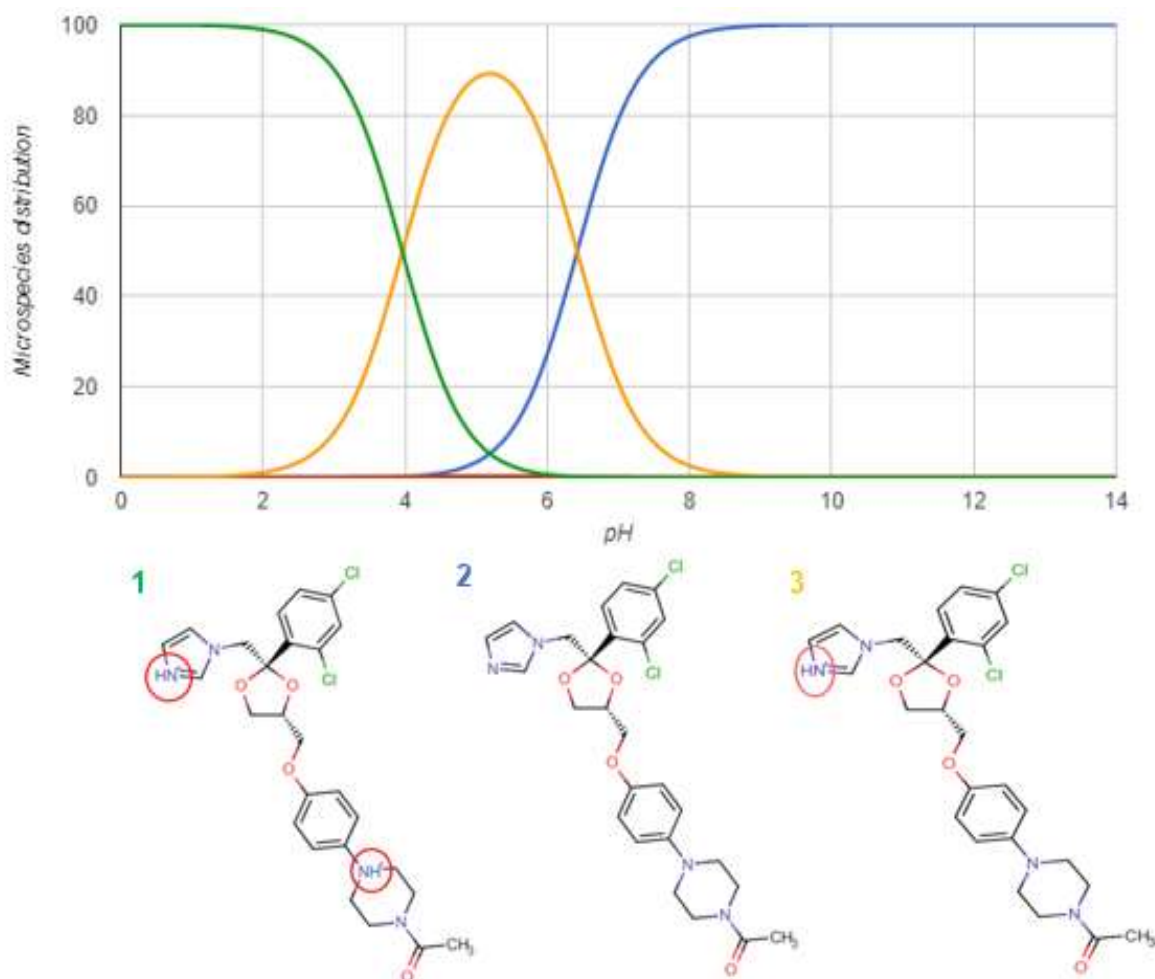


Figure 2. Microspecies distribution throughout pH range 0-14. Green line corresponds to microspecies 1 (both basic groups protonated - highlighted in red circles), blue line corresponds to microspecies 2 (neutral microspecies) and orange line corresponds to microspecies 3 (imidazole group protonated -highlighted in red circle). Images taken from <http://www.chemicalize.org/>.

Table 3. Area under the curve ($AUC_{0-\infty}$), C_{max} and T_{max} and after oral administration of ketoconazole 200 mg suspension

Parameter	Observed*	Predicted
$AUC_{0-\infty}$ ($\mu\text{g}\cdot\text{h}/\text{mL}$)	15.84 (± 7.05)	12.65
C_{max} ($\mu\text{g}/\text{mL}$)	5.04 (± 1.58)	4.73
T_{max} (h)	1.2 (± 0.5)	1.36

*Observed parameters were taken from Huang *et al.*⁴²

Human fasted - Co-administration with PPI - Hypochlorhydria

Figure 3C shows the observed and simulated plasma concentration time curve for ketoconazole in hypochlorhydria state and the plasma concentration time curve for the administration of the drug with Coca-Cola®. Both resulted in a good

match ($R^2 = 0.89$; $SSE = 0.45$; $RMSE = 0.20$; $MAE = 0.156$ and $R^2 = 0.965$; $SSE = 0.26$; $RMSE = 0.161$; $MAE = 0.125$, respectively). Values of AUC , C_{max} and T_{max} for the predicted and observed data are summarized in Table 4. All the simulated parameters were within 2-fold error of the observed pharmacokinetic parameters.

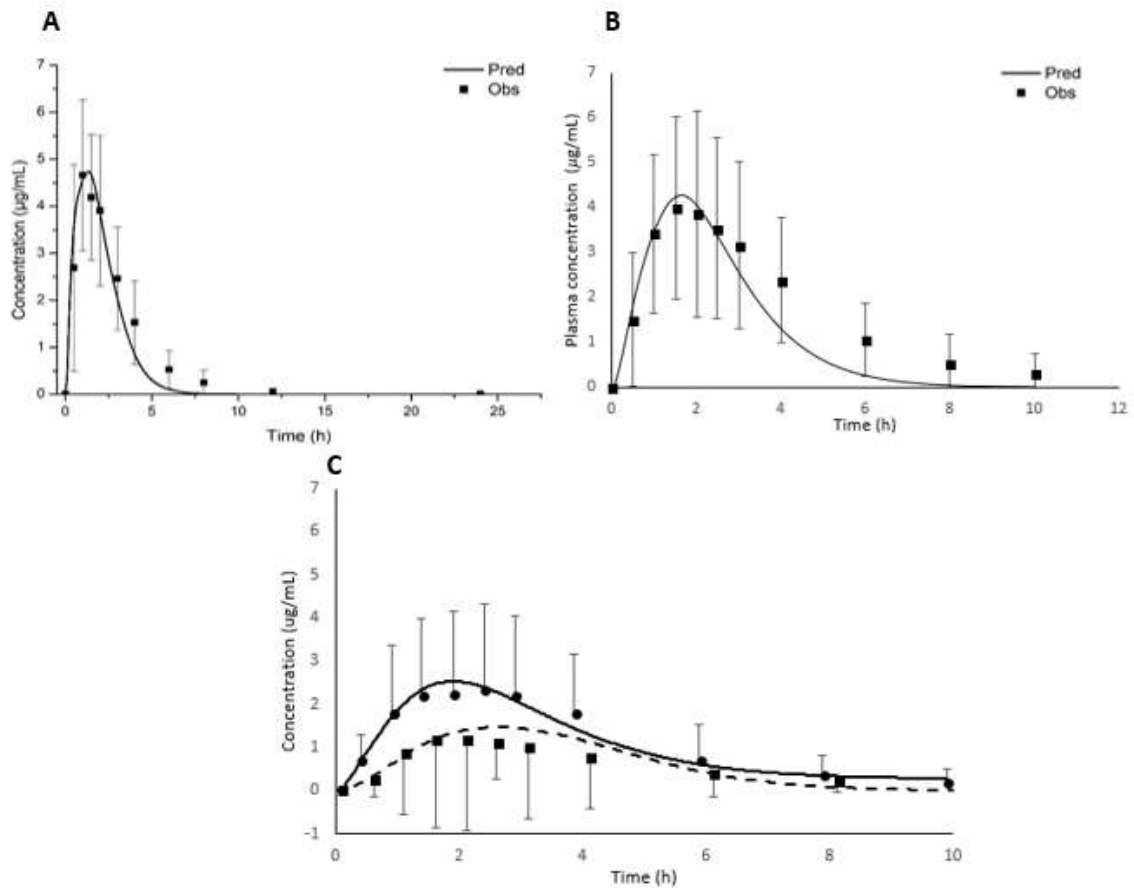


Figure 3. Plasma concentration-time profiles after administration of (A) ketoconazole 200 mg suspension; (B) ketoconazole 200 mg tablet to subjects with normal intragastric pH under a fasted condition; (C) ketoconazole 200 mg tablet to subjects with increased intragastric pH with water (predicted: dashed line; and Observed - black squares) and with Coca-Cola® (predicted: solid line and observed: black circles).

Parameter Sensitivity Analysis

Since ketoconazole is a BCS class II compound, the effect of particle size on its absorption was investigated. The PSA showed that only in a hypochlorhydric condition was ketoconazole bioavailability sensitive to changes in drug particle size (Figure 4A and B).

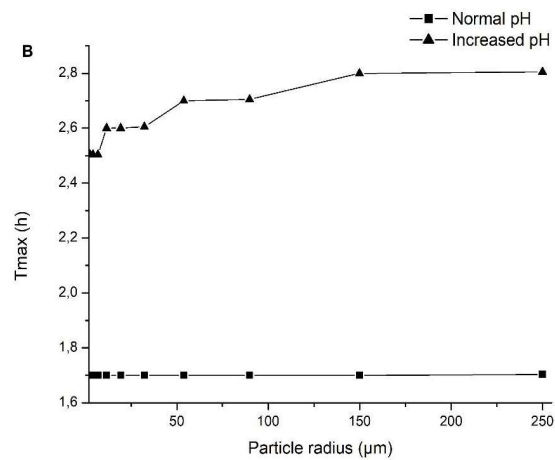
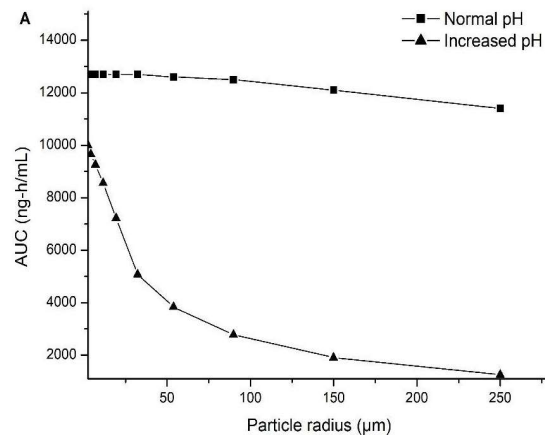


Figure 4. Parameters sensitivity analysis: influence of particle size on AUC_{0-∞}, (A) and T_{max} (B) of ketoconazole in normal (squares) and increased intragastric pH (triangle).

Solubility test

Ketoconazole had its highest solubility in SGF pH 1.2 (48 mg/mL ± 0.68); followed by Orange Juice and Coca-Cola® (2.6 mg/mL ± 0.027; 2.23 mg/mL

± 0.024 , respectively) and finally SGF pH 5.0 (0.43 mg/mL ± 0.032) in which it presented the lowest solubility. The measured equilibrium pH for the media was 2.8; 4.3; 3.62 and 5.7, respectively. The mean solubility of ketoconazole was ~ 5 times higher in Coca-Cola[®] and orange juice than its solubility in SGF at pH 5.0 and the corresponding AUC_{0-∞}, reflecting extent of systemic exposure, was increased 3-fold when the drug was administered with Coca-Cola[®] (table 4) rather than water.

DISCUSSION

Considering ketoconazole microspecies distribution (Figure 2), at normal intragastric pH (~ 1.5), the predominant microspecies (99%) has both basic groups (imidazole and piperazine) protonated, hence high solubility (Figure 2 – microspecies 1). In contrast, at pH 6.9 the predominant microspecies (75%) is neutral and poorly soluble (Figure 2 - microspecies 2) (Data taken from <http://www.chemicalize.org/>). Given that omeprazole (a PPI) reduces gastric acid secretion thus making the stomach pH much higher, it significantly impairs ketoconazole dissolution⁷. Additionally, the *in vitro* solubility test results also demonstrated a pH dependent solubility of ketoconazole.

Thus, it becomes clear that sufficient gastric acidity is of utmost importance for adequate dissolution and further absorption of the drug^{7,54}. In altered physiological states where gastric acidity isn't enough to solubilize BCS class II compounds, in this case, ketoconazole, the drug's bioavailability may be reduced. A central tenet of clinical pharmacology is the relationship between drug concentration and pharmacological/toxicological effects. Hence, the therapeutic

outcome of the given treatment will be altered as a consequence³⁸. The built *in silico* PBPK model properly predicted ketoconazole malabsorption caused by the hypochlorhydria state (Figure 3C), consistent with the primary role of gastric dissolution on the absorption of weak bases.

A common strategy used to circumvent the hypochlorhydric effect on the drug's bioavailability is to administer it with low pH drinks, such as Coca-Cola[®], in order to decrease the pH of the gastric fluid, thus increasing ketoconazole's dissolution and further absorption, as shown by Chin et al. The model was able to predict the increased bioavailability when administering the drug with Coca-Cola[®] (Figure 3C). This demonstrates the utility of *in silico* methods in further delineating different clinical strategies to avoid therapeutic failures.

As shown in Figure 4A, once sufficient gastric acidity is provided to facilitate complete ketoconazole dissolution, its absorption is not affected by particle size (squares), rather it depends on gastric transit time^{10,55}. Nevertheless, if the gastric pH is not acidic enough to completely dissolve ketoconazole in the stomach, it will depend on intestinal dissolution to be absorbed, where the particle size matters (triangles in Figure 4A). Due to its low solubility at intestinal pH, incomplete absorption will occur (showed by a smaller AUC)^{38,56}.

It is already known that particle size can influence drug absorption, mainly for BCS class II compounds⁵⁶. In API driven dissolution dosage forms, the drug's properties such as particle size and surface area will have an impact on determining drug dissolution. Thus, the drug product performance will depend on such properties in addition to the physiological environment that the drug is exposed to.

Table 4. (AUC_{0-∞}), C_{max} and T_{max} after oral administration of ketoconazole 200 mg tablet

Parameter	No hypochlorhydria		Hypochlorhydria			
	Observed*	Predicted	Observed*	Predicted	Coca-Cola [®] Observed*	Coca-Cola [®] Predicted
(AUC _{0-∞}), ($\mu\text{g}\cdot\text{h}/\text{mL}$)	17.89 (± 13.11)	12.65	3.46 (± 5.08)	6.22	11.22 (± 10.57)	18.25
C _{max} ($\mu\text{g}/\text{mL}$)	4.13 (± 1.95)	4.26	0.8 (± 1.09)	1.47	2.44 (± 1.72)	2.54
T _{max} (h)	1.5 (± 0.5)	1.67	2.9 (± 1.5)	2.61	2.2 (± 1.1)	1.9

*Observed parameters were taken from Chin *et al.*⁷

Our results corroborate that stomach and intestinal pH play a major role in ketoconazole dissolution followed by absorption process. Particle size, however, seems to play a role only under a hypochlorhydric condition.

Furthermore, both results from the PSA performed (Figure 4B) and predicted plasma concentration time curves (Figures 3B and 3C), show that the hypochlorhydria condition also results in a slower absorption rate, indicated by a longer T_{max} when compared to the one in normal intragastric pH (Table 4).

Hence, impaired absorption can occur when weakly basic drugs are administered to patients that have reduced gastric acidity, leading to a potential therapeutic failure due to subtherapeutic plasma concentrations^{3,7,57,58}.

For that reason, the development of formulations that can overcome the hypochlorhydric stomach environment is of primary importance to obtain the desired therapeutic result and efficacy^{18,20,59}.

As demonstrated by Mitra *et al.*⁵⁹ and Kou *et al.*² the use of *in silico* tools, such as GastroPlus™, can help to predict the *in vivo* performance of such formulations in humans, using PBPK and ACAT models to mimic the given patient's physiology^{20,26,28,43}. On one hand, combining *in silico* results with *in vitro* tests can be a powerful tool to select the most promising formulation to further continue studies (e.g. bioequivalence studies), on the other hand it can also be used to design different clinical approaches in order to reduce therapeutic failures^{13,19,20,37,59}. In this study we showed that rather simple computer simulations are a useful adjunctive tool when evaluating BCS II drug absorption when co-administered with a PPI. All weak bases would potentially behave in this manner; however, this could be compensated with the use of low pH beverages instead of water. Therefore, simulations come in handy to assess alternative clinical dosing approaches.

In the clinical environment PBPK models have gained much importance to enable personalized medicine and to assess drug-drug/ drug-disease interactions^{20,60-63}. For these purposes, its usefulness rely on its ability to determine the importance of subpopulations and to optimize the formulation to obtain the targeted drug plasma concentration profile⁶⁰. Nevertheless, not much attention has been drawn to the utility of computer models when designing alternative clinical approaches.

CONCLUSION

Physiologically based pharmacokinetic (PBPK) computer modeling using the software GastroPlus™ was able to accurately predict the plasma concentration vs. time profiles for ketoconazole tablets under different physiological states (normal gastric acid secretion and hypochlorhydria), capturing how well the drug would be absorbed and how the pharmaceutical product would perform under each condition. As experimentally observed, the simulated profiles showed a much lower ketoconazole absorption when the gastric acid secretion was low (hypochlorhydria) compared to individuals with a normal gastric pH. The model was also able to analyze the success of a different clinical approach (use of another beverage) showing the use of *in silico* models to support changes in clinical practice.

Thus, reliable PBPK models can be used to predict possible pharmacokinetic pitfalls, which opens up additional approaches to explore different dosing strategies in clinical practice.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare no conflicts of interest related to this work.

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