Combining species specific in vitro & in silico models to predict in vivo food effect in a preclinical stage – case study of Venetoclax

Henze, Laura J.; Koehl, Niklas J.; O'Shea, Joseph P.; Holm, René; Vertzoni, Maria; Griffin, Brendan T.

Published in:
European Journal of Pharmaceutical Sciences

DOI:
10.1016/j.ejps.2021.105840

Publication date:
2021

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Henze, L. J., Koehl, N. J., O'Shea, J. P., Holm, R., Vertzoni, M., & Griffin, B. T. (2021). Combining species specific in vitro & in silico models to predict in vivo food effect in a preclinical stage – case study of Venetoclax. *European Journal of Pharmaceutical Sciences*, *162*, Article 105840. https://doi.org/10.1016/j.ejps.2021.105840

European Journal of Pharmaceutical Sciences 162 (2021) 105840

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

Combining species specific *in vitro* & *in silico* models to predict *in vivo* food effect in a preclinical stage – case study of Venetoclax

Laura J. Henze [a], Niklas J. Koehl [b], Joseph P. O'Shea [a], René Holm [b,c], Maria Vertzoni [d], Brendan T. Griffin [a,*]

[a] School of Pharmacy, University College Cork, Cork, Ireland
[b] Drug Product Development, Janssen Research and Development, Johnson & Johnson, Turnhoutseweg 30, 2340 Beerse, Belgium
[c] Department of Science and Environment, Roskilde University, 4000 Roskilde, Denmark
[d] Department of Pharmacy, School of Health Science, National and Kapodistrian University of Athens, Athens, Greece

ARTICLE INFO

Keywords:
landrace pigs
food effect
BCS IV
in silico
FaSSIFp
in vitro
FaSSIF

ABSTRACT

The pig has been increasingly used as a reliable preclinical model for assessing and predicting the *in vivo* bioavailability of different formulation strategies. Nevertheless, differences in the composition between porcine and human intestinal fluids, may impact on the solubility and dissolution behaviour of drugs, in particular BCS II/IV drugs. Recently, a porcine fasted simulated intestinal fluid (FaSSIFp) was developed to mimic the composition in the lumen of landrace pigs under fasted state conditions. In this work, we present the utilization of FaSSIFp to compare solubility against human FaSSIF & FeSSIF and further combine species specific *in vitro* testing with *in silico* predictive modelling. Venetoclax was chosen as a model drug, representing a BCS class IV drug, with a reported clinically significant positive food effect, where bioavailability is increased up to approximately five-fold when administered with a high-fat meal. Biorelevant species specific *in vitro* testing was a promising tool for integrating *in vitro* data into *in silico* models, using FaSSIFp resulted in reliable predictions of the plasma concentration profile in fasted pigs, based on a porcine physiologically based absorption model. The porcine physiologically based absorption model was used to prospectively simulate the impact of food on the bioavailability of venetoclax. The use of luminal solubility estimates in combination with dissolution data for venetoclax, measured in species specific simulated fluids, correctly predict the observed pig plasma concentration profile and food effect. Overall, integrating species specific *in vitro* – *in silico* models led to accurate prediction of *in vivo* absorption of venetoclax in a preclinical stage, which can support guidance in early decisions of drug product development. In addition, the study further demonstrated the utility of the pig model to predict the food effects of venetoclax in humans.

1. Introduction

Preclinical bioavailability predictions of new drug candidates require a thorough understanding of factors influencing drug absorption. A key factor in dosage form development is the biopharmaceutical understanding of potential food effects of a drug product, taking into consideration not only the drug substance, but also the formulation strategy (Koziolek et al., 2019). It is crucial to assess the potential impact of food on oral pharmacokinetics early in formulation development to allow optimization of formulation design and prevent costly reformulation later in the development process (O'Shea et al., 2018). In the last decade, physiologically based pharmacokinetic (PBPK) modelling has been increasingly used to forecast formulation and food effects on *in vivo* drug plasma concentrations. The use of PBPK models during formulation development is an excellent tool for the prediction of preclinical and clinical pharmacokinetics (PK) using physiochemical and *in vitro* measurements. A key aspect for accurate predictions using PBPK models is the ability to combine accurate *in vitro* estimates of *in vivo* drug related parameters, such as luminal solubility and tissue permeability, and reliable physiological measurements, including gastrointestinal transit times, luminal volume and fluid pH (Henze et al., 2018b). Therefore, accurate estimation of drug solubility in the gastrointestinal tract is considered fundamental for better understanding of absorption limitations, in particular for BCS class II and IV compounds, where poor

* Corresponding author.
E-mail address: Brendan.griffin@ucc.ie (B.T. Griffin).

https://doi.org/10.1016/j.ejps.2021.105840
Received 15 June 2020; Received in revised form 19 March 2021; Accepted 7 April 2021
Available online 15 April 2021

solubility is likely to be a rate limiting step in absorption (Walsh et al., 2017). Physiochemical characterisation of human intestinal fluids is well described (Augustijns et al., 2014) and the development of fluids to simulate either gastric (fasted state simulated gastric fluid, FaSSGF) or intestinal (fasted state simulated intestinal fluid, FaSSIF) contents is well documented (Jantratid et al., 2008; Vertzoni et al., 2005). These established biorelevant media, are routinely used for dissolution and solubility studies during drug development (Bergstrom et al., 2014; Klein, 2010). While these media have proved suitable for humans, FaSSIF cannot be used for the simulation of intestinal contents of preclinical models, due to key differences in animal and human intestinal fluid characteristics (Arndt et al., 2013; Henze et al., 2020; Sjogren et al., 2014). Therefore, species specific intestinal media have been developed to simulate preclinical animal gastrointestinal conditions, with the aim to improve predictions and interpretation of preclinical results. Arndt *et al.* developed different canine biorelevant media simulating fasted gastric and intestinal conditions in dogs (Arndt et al., 2013). More recently a porcine biorelevant medium was established, based on fasted intestinal content characteristics of pigs (Henze et al., 2020). Both media are specially adapted to reflect species specific properties in the gastrointestinal tract, such as pH, buffer capacity, osmolality, surface tension and bile and phospholipid concentrations. Combining species specific *in vitro* testing with *in silico* modelling, may give early insights in preclinical development of drug behaviour after oral administration and can be employed to allow drug formulation characterisation in advance of *in vivo* testing (Arndt et al., 2013). To date, only the canine media have been used to explore the predictability of plasma concentration profiles in dogs, combining the species specific *in vitro* testing with *in silico* modelling (Walsh et al., 2017). The observed data indicated that the solubility in canine biorelevant media was significantly higher compared to human FaSSIF, in particular for weak acids. Using a model drug ('compound free acid 6'), the pharmacokinetics of amorphous and crystalline formulations in beagle dogs was predicted using *in silico* modelling. The simulations showed that using human FaSSIF the observed drug plasma concentrations were under predicted for both formulations, while the canine biorelevant media clearly improved the predictions.

Recently, we have developed a protocol for predicting food effect using a pig model, utilizing a standard high-caloric, high-fat meal to mimic postprandial conditions present in human food effect studies (FDA, 2002; Henze et al., 2019). Given the dual priorities in early drug development phases of developing bioenabling formulations to address solubility limitations and assessing the impact of food on bioavailability as early as possible, is it logical that these decisions are made in parallel, rather than in sequence. Hence, reliable preclinical models are important to assess the interplay of food and drug formulations, as well as providing insights to the optimal formulation approach for poorly soluble drugs.

The main objective of this study, was to assess the suitability of species specific *in vitro* solubility and dissolution data to predict the drug absorption profile of venetoclax in pigs. A secondary aim of this study was to assess the reliability of the combined *in vitro-in silico* porcine model to predict the post-prandial drug absorption profile by comparison to the *in vivo* absorption observed in fed state in pigs. Venetoclax, a BCS Class IV drug, was chosen as a model drug on the basis of its profound food dependent bioavailability reported in humans (Salem et al., 2016). These properties lead to challenges during formulation development and prediction of the absorption behaviour under postprandial conditions. Therefore, this study provides new insights on the use of *in vitro* and *in silico* tools in preclinical development using a model drug with poor biopharmaceutical properties to predict food effects in humans.

2. Materials and methods

2.1. Chemicals and materials

Venetoclax was purchased from Kemprotec Ltd. (UK). Venclyxto® 100 mg tablets were commercially sourced from local pharmacies. Lipoid E PC S (Phosphatidylcholine) was obtained from Lipoid GmbH (Germany), sodium taurodeoxycholate; sodium hydroxide (NaOH) pellets; chloroform; sodium chloride (NaCl); sodium dihydrogen phosphate monohydrate; sodium oleate were purchased from Sigma Aldrich (Ireland) and sodium taurocholate was ordered from Thermo Scientific Ltd., Alfa Aesar (UK). Fasted state simulating intestinal fluid (FaSSIF) and fed state simulating intestinal fluid (FeSSIF) powder was kindly donated by biorelevant.com (UK). All food components used in preparing FDA standard breakfast were purchased commercially. Water was produced using a MilliQ system (Merck KGaA, Germany). All other chemicals and solvents were of analytical grade or HPLC grade, respectively, and were purchased from Sigma–Aldrich (Ireland) and used as received.

2.2. Biorelevant solubility and dissolution

Solubility was measured *ex vivo* in pig intestinal fluids (PIF). Pig intestinal contents have been collected in previous studies and upon collection they were frozen (-80°C) until further analysis (Henze et al., 2019). On the day of the solubility experiment all samples were brought to room temperature. Fluid samples were pooled by taking 3 mL from each sample (total 6 samples) to create the PIF samples. FaSSIF and FeSSIF were prepared as outlined in the instructions by biorelevant.com. FeSSIF was used directly, whereas FaSSIF was left at room temperature for 2 hours prior to the solubility studies. Porcine Fasted State Simulated Intestinal Fluid (FaSSIFp) was prepared as recently described (Henze et al., 2020). The composition of FaSSIFp is summarized in Table 1. Solubility studies were carried out by the addition of excess venetoclax to biorelevant media using a shake flask method (200 shakes/min) with

Table 1
Composition and physiochemical properties of the medium to simulate the porcine fasted state small intestine – Fasted State Simulated Intestinal Fluid porcine (FaSSIFp)

Composition	FaSSIFp	mM
Buffer	Sodium dihydrogen phosphate monohydrate	35.71
	Sodium hydroxide	13.62
	Sodium chloride	135.32
Bile salts	Sodium taurocholate	5.25
	Sodium taurodeoycholate	9.72
Phospholipids	Lecithin	0.20
Fatty acid	Sodium oleate	2.82
pH	7.0	
Buffer capacity [mmol $l^{-1}\Delta pH^{-1}$]	19.4	
Osmolality [mOsm kg^{-1}]	387	

a shake time of 24 h at 37°C. Samples were taken at 3 h, 6 h and 24 h and added to 1.5 mL centrifuge tubes. Samples were centrifuged at 11,000 rpm for 10 min (Mikro 200 R, Hettich GmbH, Germany). The supernatant was transferred to a new tube and 2 mL of acetonitrile was added (PIF solubility) and centrifuged again under the same conditions. The resultant supernatant was analysed using HPLC after appropriate dilution with mobile phase. All samples were run in triplicate.

Biorelevant dissolution was carried out using the dissolution apparatus USP II (Erweka DT600, Erweka GmbH, Germany) at 75 rpm. Tests were performed using 500 mL of FaSSIF, FeSSIF or porcine biorelevant media at 37 ± 0.5 °C. Experiments were performed in triplicate. Venetoclax tablets (Venclyxto® 100 mg tablets) were placed in the dissolution medium, and samples of 4 mL were withdrawn at 5, 10, 15, 20, 30, 45, 60, 90, 120 and 180 min, immediately followed by addition of an equal volume of fresh, pre-warmed medium. The withdrawn samples were filtered through a 0.45 µm cellulose acetate membrane filter (VWR International), discarding the first 2 mL. The resultant filtrate was visually assessed as being clear and free from particles. 100 µL of sample was immediately diluted with 900 µL of mobile phase and analysed using HPLC.

2.3. Quantitative analysis of venetoclax solubility and dissolution samples

The concentrations of venetoclax from solubility/ dissolution experiments were determined using a validated HPLC-UV method (Koehl et al., 2020). In brief, the limit of detection (LOD) was determined to be 19.52 ng/mL and the limit of quantification (LOQ) was 65.08 ng/mL The variability around the LOQ is 5.2 %. The reproducibility of the method at 500 ng/mL, 1250 ng/mL, 2000 ng/mL, 3000 ng/mL, 4000 ng/mL, 9500 ng/mL and 12500 ng/mL, expressed as the inter-day coefficient of variation was 5.19 %, 3.66 %, 4.96 %, 4.73 %, 4.18 %, 4.00 % and 3.67 % respectively. Linearity was demonstrated between the range of 50 ng/mL to 12.5 µL/mL. Samples were analysed using an Agilent 1200 series HPLC system comprised a binary pump, degasser, column oven, autosampler and variable wavelength detector. Data analysis was carried out with EZChrom Elite version 3.2. Venetoclax was separated with a Zorbax Eclipse Plus-C18 column (5 µm, 4.6 mm x 150 mm) including a Zorbax Eclipse Plus-C18 guard column (5 µm, 4.6 mm x 12.5 mm) at 40 °C. The mobile phase consisted of acetonitrile with 0.5 % TFA and Water with 0.5 % TFA (53:47) and was used at a flow rate of 1 mL/min. 20 µL samples were injected and the detection wavelength was 290 nm. The limit of detection (LOD) was 20 ng/mL and the limit of quantification (LOQ) 65 ng/mL determined using the standard error of y-intercept according to ICH Q2 (ICH, 2005).

2.4. In vivo oral bioavailability study in pigs

The study was carried out under the licence issued by the Health Products Regulatory Authority (HPRA), Ireland, as directed by the Cruelty to Animals Act, Ireland and EU Statutory Instruments (Licence number AE19130/P058). Local University ethical committee approval was obtained. The study was a non-randomised, three-way crossover design (I. fasted state, II. Fed state, III. intravenous administration) where five male landrace pigs (15–17 kg) were sourced locally and housed individually at the University's Biological Services Unit. Throughout the study pigs were fed approximately 175 g of standard weanling pig pellet feed twice daily. In the fasted leg of the study the final feed of 175 g was given 24 h prior to dosing. As part of the study design any remaining food was removed 16 h before dosing, however, no food remained at this point in any of the groups. In the fed state pigs were fed a half portion of a standard high-caloric, high-fat FDA breakfast (444 kcal, 315 g, one slice of bacon, one slice buttered toast, one fried egg, 118 mL whole milk, 60 g hashed brown potatoes). The mass of FDA breakfast fed equated to approximately 18 – 20 g/kg of body weight.

On day one, an indwelling intravenous catheter was inserted into the jugular vein via an ear vein under general anaesthesia, as previously described (Framstad T., 2000; Pairis-Garcia et al., 2014; Swindle, 2010, 2016; Swindle and Smith, 2013a). Following an overnight fast on day three, oral formulation of 100 mg venetoclax (Venclyxto®) was administered with the aid of a dosing device, after which the pigs received 50 mL of water via a syringe. Under fed state conditions, pigs received the meal 30 minutes prior to oral dosing. After dosing, pigs were returned to their pens. Blood samples (4 mL) were collected at time zero (pre-dosing) and 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 24 h post-dosing. Water was restricted for 3 h post dosing and all pigs were fed 175g of pig feed 8 h post dosing. All blood samples were collected in heparinised tubes (Sarstedt, Germany) and immediately centrifuged at 3220 *g* for 5 min at 4 °C (Eppendorf 5810r swinging bucket rotor centrifuge, Eppendorf AG, Hamburg, Germany). Plasma was collected and stored at −80 °C. A six-day washout period was used between each phases. All animals remained in good health throughout the study.

The intravenous venetoclax formulation contained 25 % Tween 80 in water for injection, which was administered over 5 minutes, blood samples were collected at 5, 15, 30, 45 min, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24 h, centrifuged and collected plasma was stored at −80 °C.

2.5. Bioanalysis of venetoclax

The plasma concentrations of venetoclax were determined by reversed phase HPLC. The Agilent 1260 series system comprised a binary pump, degasser, temperature controlled autosampler, column oven and diode array detector. The system was controlled, and the data analysed with EZChrom Elite version 3.3.2. A Zorbax Eclipse Plus-C18 column (5 µm, 4.6 mm x 150 mm) with a Zorbax Eclipse Plus-C18 guard column (5 µm, 4.6 mm x 12.5 mm) was used for the separation of venetoclax. The mobile phase consisted of water and acetonitrile with 0.5 % TFA (47:53 v/v) and was used at a flow rate of 1.0 mL/min. The sample and column temperature were set at 5 °C and 40 °C, respectively, and the detection wavelength was set to 250, 290 and 316 nm. Venetoclax was extracted from the plasma samples by liquid-liquid extraction. To 500 µL of the plasma 50 µL vemurafenib (internal standard dissolved in acetonitrile) and 450 µL of acetonitrile was added. The mixture was mixed thoroughly, and 1 mL of ethyl acetate was added followed by mixing for 15 sec. The mixture was centrifuged at 25 °C, 11,500 *g* for 5 minutes. 1.5 mL of the supernatant was recovered and transferred to a new tube. The supernatant was dried at 60 °C under a nitrogen stream. To the remaining plasma sample 1 mL of ethyl acetate was added and the mixture was thoroughly mixed and centrifuged using the same condition as above. 1 mL supernatant was recovered and transferred to the same tube as above. After drying of the supernatant, the residues were reconstituted in 100 µL mobile phase (excluding TFA) followed by centrifugation at 25 °C, 11,500 *g* for 5 minutes. 50 µL of the supernatant was injected for analysis. The LOD and LOQ in plasma by this method was 6 ng/mL and 20 ng/mL, respectively, determined using the standard error of y-intercept according to ICH Q2 (ICH, 2005). Linearity was confirmed between 25 ng/ml and 2.5 µg/ml.

2.6. Pharmacokinetic analysis

Exposure after oral administration was estimated by calculating the area under the plasma concentration curve (AUC) for 8 h and 24 h post dosing for venetoclax by using PKPlus (Gastroplus.9.5). AUC and mean residence time (MRT) were determined from non-compartmental analysis. The peak plasma concentrations (C_{max}) and the time for their occurrence (t_{max}) were noted directly from the individual plasma concentration versus time profiles. The absolute bioavailability (F_a) of venetoclax was calculated according to Eq. 1. All pharmacokinetic parameters are reported as mean ± SD.

$$F_a = \left(\frac{AUC_{oral}}{AUC_{i.v.}}\right) * \left(\frac{Dose_{i.v.}}{Dose_{oral}}\right) \quad (1)$$

Mean absorption time (MAT) was calculated from intravenous and oral MRT, according equation 2:

$$MRT_{po} = MAT + MRT_{iv} \quad (2)$$

Food effect was calculated using the fold difference (FD) in the AUC in fed vs the fasted state using equation 3:

$$FD = \left(\frac{AUC_{fed}}{AUC_{fasted}}\right) \quad (3)$$

Fold differences (FD) are presented, where possible, as mean FD ± standard error of the fold difference (SE_{FD}) as calculated by equation 4:

$$SE_{FD} = FD \times \sqrt{\frac{SE_{fed}{}^2}{AUC_{fed}{}^2} + \frac{SE_{fasted}{}^2}{AUC_{fasted}{}^2}} \quad (4)$$

Where FD is the mean fold difference in food effect, AUC_{fed} and AUC_{fasted} are the represent the mean AUC in the fed and fasted states, respectively, and SE_{fed} and SE_{fasted} represent the standard errors corresponding to these values, respectively.

2.7. In silico predictive modelling

In silico absorption modelling was conducted using GastroPlus™ (vers. 9.7, Simulations Plus, Lancaster, Ca.). The ADMET Predictor™ module was used to estimate venetoclax physiochemical characteristics. Further drug specific data was included in the drug database based on published literature (i.e. pKa, experimental permeability values, metabolic pathways, log*P*, amorphous solubility) (Emami Riedmaier et al., 2018; FDA, 2015), and are summarized in Table S1 (supplementary data). Biorelevant solubility were changed to those measured *in vitro*. The z-factor was established by fitting individual dissolution profiles in FaSSIF, FeSSIF and porcine biorelevant media (FaSSIFp) as appropriate for the model generated. Pharmacokinetic parameters and bioavailability were fitted to a one-, two- and three-compartment model using PKPlus™ module, with goodness of fit assessed using the Akaike Information Criterion (AIC) and comparison to previously published venetoclax pharmacokinetic models (Jones et al., 2016). Simulations were set to 24 h using the minipig physiology fasted ACAT™ model, and incorporated a feeding step at 8 h post dosing. Dose was set to 100 mg and dose volume was 50 mL. The minipig physiology fed ACAT™ model was modified prior to conducting simulations. Specifically, gastric transit time was changed to 5.0 h, based on recently published data (Henze et al., 2018a; Henze et al., 2019). Further modifications included: adjustment of the food intake, food type was aligned with the kcal pigs received during the *in vivo* studies (FDA style breakfast). Gastrointestinal fluid volumes were adjusted to 180 mL in the stomach compartment and 120 mL in the small intestinal compartment in the fasted and 575 mL and 262 mL in the fed state, based on post-mortem collected gastrointestinal content samples (in-house data). All other values were kept at default values. Simulated profiles were compared to the mean plasma profiles for fasted and fed state conditions. Percent prediction errors (% PE) were calculated for each simulation relative to the observed data, according to equation 5 (Pathak et al., 2017).

$$\%\ PE = \left(\frac{predicted\ mean - observed\ mean}{observed\ mean}\right) * 100 \quad (5)$$

2.8. Statistical analysis

The statistical analysis was performed using a paired t-test to determine statistical significance ($p < 0.05$) of calculated *in vivo* bioavailability and pharmacokinetic results. *In vitro* solubility data were tested for significance ($p < 0.05$) using a two-tailed, independent sample t-test, assuming Gaussian distribution and equal variance. All statistical analyses were performed using GraphPad Prism version 5.

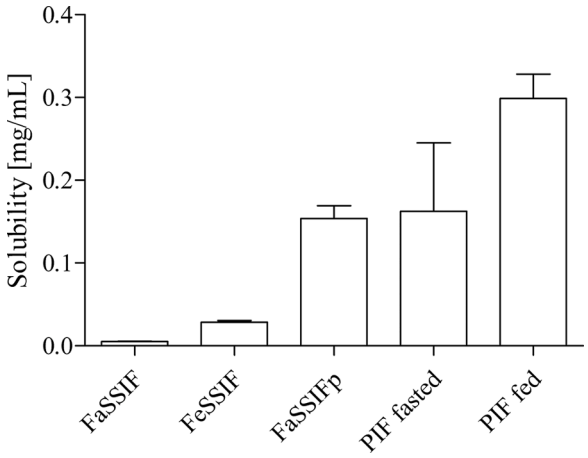

Fig. 1. Solubility of venetoclax in fasted state simulated intestinal fluid (FaSSIF), fed state simulated media (FeSSIF), fasted stated simulated porcine intestinal fluid (FaSSIFp), porcine intestinal fluids (PIF) in the fasted and in the fed state conditions (n=3, mean ± SD).

3. Results

3.1. Biorelevant solubility in porcine intestinal media

The solubility of venetoclax was measured in *ex vivo* samples of porcine intestinal fluids (PIF) and compared to the solubility measured in three different biorelevant media, the results of which are summarized in Fig. 1. Biorelevant media representing both the fasted state (FaSSIF) and postprandial (FeSSIF) conditions in human intestine were used. In addition, a species specific biorelevant media representing the fasted intestinal state in landrace pigs (FaSSIFp) was used as predictor of *in vivo* solubility at the absorptive site.

The solubility of venetoclax measured in FaSSIFp was 154 ± 15 μg/mL, which is higher compared to human FaSSIF (5.2 ± 0.3 μg/mL) and can be attributed to the the higher bile salt concentrations in FaSSIFp, which is specifically designed to mimic pig intestinal conditions in the fasted state (Henze et al., 2020). The solubility in human biorelevant media of venetoclax increased from 5.2 ± 0.3 μg/mL in FaSSIF to 28.4 ± 2.2 μg/mL in FeSSIF, reflecting a 5.5 ratio FeSSIF/FaSSIF, which correspond to the reported clinical food effect of venetoclax (5- fold increase AUC_{fed}/AUC_{fasted}) (Salem et al., 2016).

The measured solubility of venetoclax in *ex vivo* pig intestinal fluids (PIF) under fasted conditions was 163 ± 82 μg/mL and 299 ± 29 μg/mL in the fed state, reflecting a ratio of 1.8 PIF_{fed}/PIF_{fasted}. Therefore, while

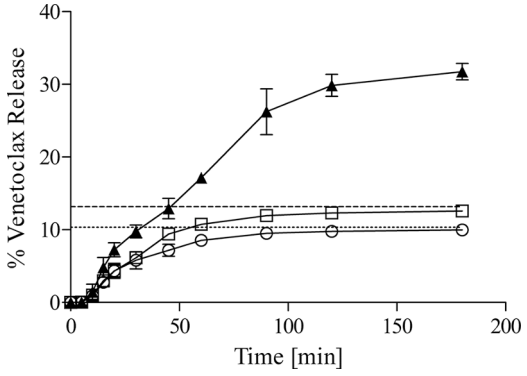

Fig. 2. Biorelevant dissolution of venetoclax 100 mg tablets (Venclyxto®) in simulated intestinal media, (o) FaSSIF, ([]) FeSSIF, (▲) FaSSIFp. Dotted line indicates the reported amorphous solubility of venetoclax in FaSSIF and dashed line indicates the reported amorphous solubility of venetoclax in FeSSIF (Emami Riedmaier et al., 2018) (n=3, mean ± SD).

a trend towards higher solubility was observed in post prandial luminal fluids, overall the ratio was lower than the ratio observed in FeSSIF/ FaSSIF. Interestingly the solubility of venetoclax in porcine FaSSIFp (154 ± 15 μg/mL) closely matched the solubility in *ex vivo* pig fasted intestinal fluids (163 ± 82 μg/mL), which supports the suitability of using simulated porcine media to predict luminal drug concentrations.

3.2. Biorelevant dissolution under simulated fasting and fed state conditions

The dissolution characteristics of venetoclax in porcine biorelevant media (FaSSIFp) were compared to the dissolution performance using human biorelevant media (FaSSIF & FeSSIF), as presented in Fig. 2.

The dissolution profile obtained from FaSSIFp, showed a marked increase in the extent of dissolution relative to those observed in human FaSSIF, with release of 32.1 % after 3 h. Drug release under human simulated fasting conditions was low, with approximately 10 % dissolution observed after 3 h. This increased to 12.6 % release after 3 h using FeSSIF media. The drug release in human biorelevant media was compared to the previously reported values of amorphous solubility of venetoclax in FaSSIF and FeSSIF, and the drug concentration observed after 3 h of dissolution were closely aligned with amorphous solubility of the drug (Fig. 2) (Emami Riedmaier et al., 2018).

3.3. Validation of in silico model with biorelevant in vitro input

In order to assess the disposition kinetics of venetoclax in pigs, a venetoclax formulation containing 25 % Tween 80 in water for injection, 17 mg of venetoclax was administered intravenously (i.v.). The venetoclax plasma concentration time profile obtained after i.v. administration is shown in Fig. 3. These data were fitted to one- (AIC = -6.8863), two- (AIC = -42.7994) and three-compartment (AIC = -56.674) models. Overall, it was decided to use a two-compartment given that the model was found to adequately fit the observed data, with limited improvement in using the more complex three-compartment model. Furthermore, a two compartment model had been successful employed to model previously for pharmacokinetic analysis of venetoclax(Jones et al., 2016). The key pharmacokinetic parameters are summarised in Table 2. The observed volume of distribution was 0.11 ± 0.02 L/kg, which was marginally lower to values reported in humans (0.21 L/kg) (Emami Riedmaier et al., 2018). The total clearance of venetoclax in this study was determined to be 0.13 ± 0.02 L/(h*kg).

The mean plasma concentration profiles obtained after oral administration of 100 mg Venclyxto® (venetoclax) to fasted pigs are presented in Fig. 3. In the fasted state, the median time to reach peak concentrations (t_{max}) was 12 h (range: 8 – 12 h) due to a secondary peak after 8 h post dosing. This secondary absorption phase most likely reflects a post prandial phase, as pigs were allowed access to food (after an 8 h fasting period post dosing), which resulted in an increased drug absorption at this 8- 12 h period. Furthermore, mean absorption time (MAT) was affected by this secondary absorption phase, resulting in a longer MAT under fasted conditions, ranging from 7.4 to 15.3 h (median 10.0 h), compared to the fed state.

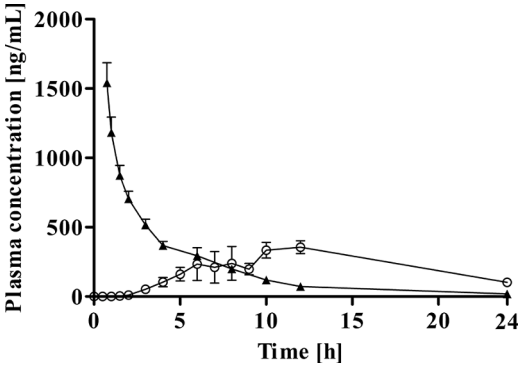

Fig. 3. Plasma concentration time profile following intravenous administration of 17 mg of venetoclax to landrace pigs (▲); Plasma concentration time profile from 0 – 24 h of 100 mg venetoclax in landrace pigs under fasted (o) conditions. (mean ± SEM, n= 5).

Table 2
Summary of pharmacokinetic parameters following intravenous administration of 17 mg of venetoclax and oral administration of 100 mg to fasted pigs (mean ± SD, n=5).

IV Pharmacokinetic parameters		Oral Pharmacokinetic parameters fasted state	
V_c (L/kg)	0.11 ± 0.02	C_{max} (μg/mL)	0.43 ± 0.19
CL (L/(h*kg)	0.13 ± 0.02	t_{max} (h) [a]	12.0 (8.0 – 12.0)
k_{el} (h^{-1})	1.17 ± 0.17	$AUC_{0-\infty}$ (μg·h/mL)	5.5 ±1.2
k_{12} (h^{-1})	1.64 ± 0.32	$Fa_{0-\infty}$ (%) [b]	12.9 ± 3.7
k_{21} (h^{-1})	0.43 ± 0.11	MAT (h) [a]	10.0 (7.4 – 15.3)
$AUC_{0-\infty}$ (μg·h/mL) [c]	0.44 ± 0.05	MRT p.o. (h) [a]	14.4 (11.0 – 19.6)

[a] median (range)
[b] $F_{0-\infty}$ = (AUC(oral)$_{0-\infty}$/ AUC(iv)$_{0-\infty}$)*(Dose(iv)/Dose(oral)
[c] IV dose corrected

Integration of species specific *in vitro* methods into *in silico* models to predict *in vivo* drug levels of venetoclax have been generated by using GastroPlus™ software provided by Simulations Plus, Inc., Lancaster, California, USA. Modelling of plasma venetoclax concentrations for Venclyxto® 100 mg tablets, including *in vitro* solubilities and dissolution kinetics under porcine biorelevant conditions and PKPlus™ fitted pharmacokinetic estimates led to an absorption model which accurately predicts the observed data in the fasted state.

The venetoclax plasma profiles were modelled for Venclyxto® 100 mg tablets, incorporating *in vitro* solubilites (in PIF and FaSSIFp) and dissolution outcomes under biorelevant conditions (in FaSSIFp) and PKPlus™ fitted pharmacokinetic estimates using the default mini-pig physiological fasted ACAT™ model. Initial model estimates resulted in an inadequate prediction of the observed data in the fasted state (Fig. 4 A). While the default gastric emptying time (GET) in the mini-pig physiological fasted ACAT™ model is 0.70 h, corresponding to the average time taken for material to pass through the stomach compartment. Based on our previous studies, using paracetamol as a marker for gastric emptying the fasted GET in landrace pigs was estimated to be 5.0 h (Henze et al., 2019). Therefore, a slower gastric emptying rate in fasted pigs was incorporated into the model, which resulted in delayed absorption phase (Fig. 4 B). Further adjustments of the default mini-pig physiological fasted ACAT™ model were deemed necessary to represent gastrointestinal conditions in landrace pigs. Based on gastrointestinal content samples collected post-mortem luminal fluid volumes were adjusted to 180 mL stomach compartment and 120 mL small intestinal compartment based on in-house data and published data (Henze et al., 2019). This resulted in a prediction illustrated in Fig. 4 C, which accurately predicts the initial absorption phase up to 8 h. However, the prolonged absorption phase observed *in vivo*, is not adequately modelled, with the result that C_{max} is lower in the predicted profile. ($Cmax_{predicted}$ 0.23 μg/mL versus $Cmax_{observed}$ 0.36 μg/mL). The distinct second peak observed in the fasted plasma concentration profiles *in vivo* occurred at 8 - 12 h, which matches with the time pigs were allowed access to food (i.e. 8 h post dosing). This observation is similar to previous reports observed for fenofibrate in landrace pigs and is suggestive of a post-prandial induced secondary absorption phase, with absorption of drug remaining in the gastrointestinal tract occurring in response to ingestion of food (O'Shea et al., 2015). This prolonged absorption phase is not observed in the models used to generate the simulated plasma profiles in Fig. 4 A-C. Therefore, a second feeding stage at 8 h post dosing was integrated into the physiological fasted ACAT™ model (Fig. 4 D). The combination of the prolonged gastric

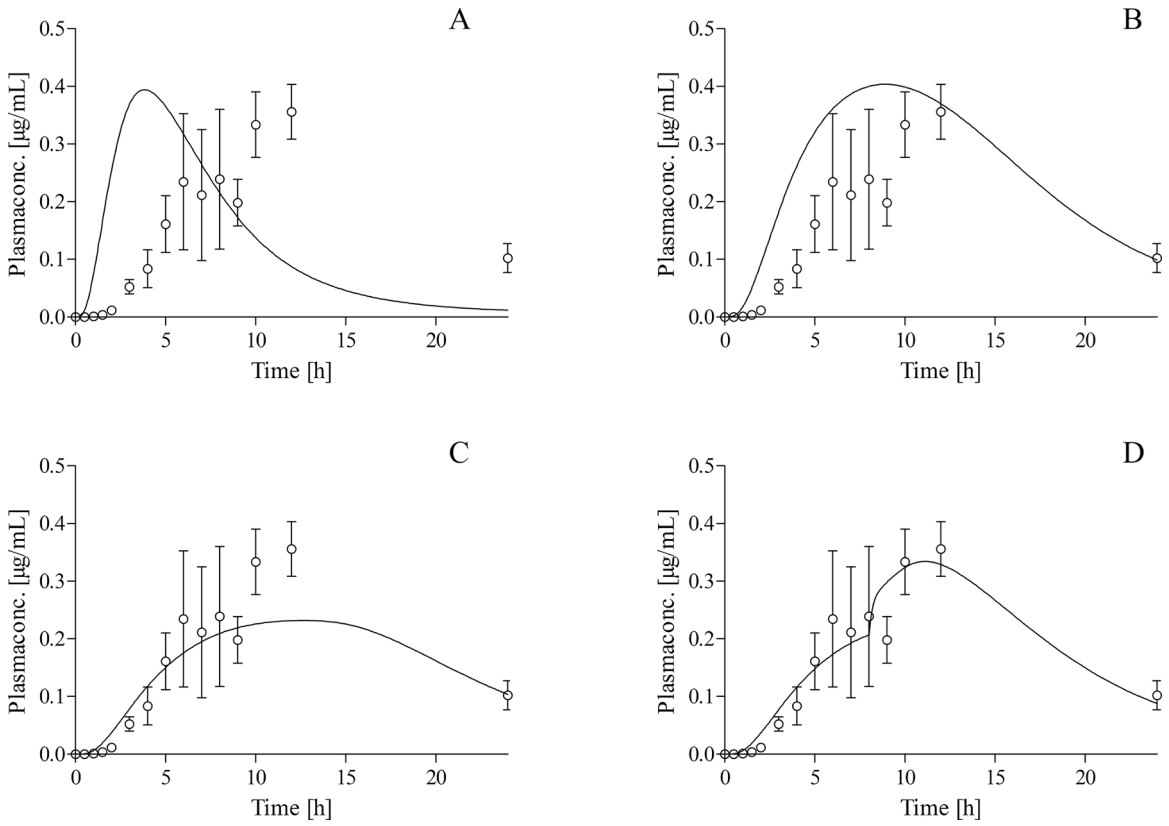

Fig. 4. GastroPlus™ in silico model of plasma venetoclax concentration vs time profile compared to observed in vivo pig data, line indicates the predicted data, (o) indicates the observed data (n= 5, mean ± SEM); A: standard mini-pig physiological fasted ACAT™ model; B: mini-pig physiological fasted ACAT™ model with a delayed gastric emptying (5.0 h); C: landrace specific model with a delayed gastric emptying and gastrointestinal volumes adjustment; D: landrace specific model with a delayed gastric emptying, gastrointestinal volumes adjustment and a incorporated feeding step at 8 h post dosing.

transit, the reduction of gastrointestinal fluid volume and the introduction of a modelled feeding step 8 hours post dose appears to improve the overall correlation of the simulated and observed plasma profiles. A positive correlation was observed between predicted and observed plasma concentrations. This model predicted bioavailability in the fasted state to be 11.6 % (% PE -10 %) with a Cmax of 0.33 µg/mL at 11 h (% PE – 6 %).

3.4. Applying an integrated in vitro- in silico approach to predict a food effect of venetoclax in pigs

The mean plasma concentration profiles obtained after oral administration of 100 mg Venclyxto® (venetoclax) to fed pigs are presented in Fig. 5. The main pharmacokinetic parameters are summarized in Table 3.

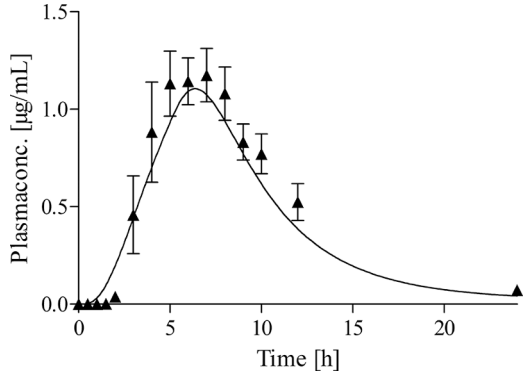

Fig. 5. GastroPlus™ in silico model of plasma venetoclax concentration vs time profile compared to observed in vivo pig data, solid line indicated the predicted fed state profile, (▲) indicated the observed data (n=5, mean ± SEM).

Table 3
Pharmacokinetic parameters of venetoclax after oral administration of 100 mg per animal under postprandial conditions (mean ±SD, n=5).

Pharmacokinetic parameters		
	Fed $_{observed}$	Fed $_{predicted}$
C_{max} (µg/mL)	1.24 ± 0.29	1.11
t_{max} (h) [a]	6.0 (4.0 – 8.0)	6.4
$AUC_{0-\infty}$ (µg·h/mL)	12.5 ± 3.2 [c]	9.53
$Fa_{0-\infty}$ (%) [b]	28.7 ± 7.5 [c]	22.2
Food effect $_{0-\infty}$ [d]	2.3 ± 0.5, (1.79 – 3.20)	1.86

[a] median (range)
[b] $F_{0-\infty} = (AUC(oral)_{0-\infty}/ AUC(iv)_{0-\infty})*(Dose(iv)/Dose(oral)$
[c] significant difference to the fasted equivalent parameter ($p < 0.05$)
[d] calculated according to equation 3, reported as mean values of the individual fed/fasted ratios

In the fed state the absorption of venetoclax was significantly higher compared to the fasted state. $AUC_{0-\infty}$ increased from 5.5 ± 1.2 µg·h/mL in the fasted state to 12.5 ± 3.2 µg·h/mL in the fed state. The absolute bioavailability of 28.7 ± 7.5 % in the fed state compared to 12.9 ± 3.7 % in the fasted state, reflecting a 2.3 ± 0.5-fold increase ($p<0.05$) after administration of the FDA style high-fat, high-caloric breakfast. Looking at the 0- 8 h post dose period (i.e. AUC_{0-8h}) indicated an even more pronounced 8.4 ± 4.0-fold increase in bioavailability between fed and fasted state conditions.

The combination of species specific *in vitro* and *in silico* methods to predict *in vivo* drug levels in the fed state have been used to assess the potential impact of food on the bioavailability of venetoclax. Modelling

of plasma venetoclax concentrations for Venclyxto® 100 mg tablets, included luminal solubility of venetoclax under fasted and fed state condition (PIF_{fasted} & PIF_{fed}) and dissolution kinetics under porcine biorelevant conditions (FaSSIFp), which lead to an absorption model which accurately predicts the observed *in vivo* drug levels in the post-prandial state.

The porcine *in silico* model predicted a 22 % increase in oral bioavailability in the fed state, and an overall predicted food effect of 1.86 was predicted, which compared well with the range of values found *in vivo* (1.79 – 3.20). The *in silico* predictions are therefore in line with observations *in vivo* for absolute bioavailability in the fed state (% PE -23 %). Furthermore, the predicted C_{max} of 1.11 μg/mL at 6.4 h compares to C_{max} 1.18 μg/mL at 7.0 h *in vivo* (% PE -10 %).

A crucial input parameter for this model, mimicking the postprandial absorption of venetoclax, was the solubility measured in pig intestinal fluid samples under fasted and fed conditions (PIF), which was combined with the dissolution kinetics obtained using FaSSIFp. A sensitivity analysis was conducted using three-dimensional surface response plots, examining the effect of intestinal solubility (based on the bile salt solubilisation ratio), as well as dissolution kinetics (based on the dissolution parameter, *z*- factor) on simulated plasma profiles. The increase of intestinal solubility/ bile salt solubilisation ratio leads to an increase in absorption, in comparison the effect of the *z*- factor as input parameter was less significant (supplementary data, Fig. S1).

4. Discussion

During drug development there is a clear need for preclinical species specific *in vitro* and *in silico* tools to provide early forecasts of drug formulation performance *in vivo*. Therefore, improvements of *in vitro- in silico* models to predict *in vivo* absorption will address dual goals of (i) developing optimal formulation strategies to improve drug absorption, and (ii) predicting impact of food on oral drug product performance early in the product lifecycle. Developing biopharmaceutics tools that link formulation design to overcome solubility limitation and reduce food effect are key to avoid costly re-formulation in later stage of clinical/ commercial development. Accordingly, integrating species specific *in vitro* tools with *in silico* models provide for more informed decision making on the formulation properties and possible food effects in a preclinical setting.

As a BCS class IV drug, venetoclax displays poor solubility and permeability. This class of drugs typically exhibit dissolution or solubility limited absorption (Emami Riedmaier et al., 2018). Therefore, an estimation of drug solubility in the gastrointestinal tract is considered critical to better understand limitations of their absorption. In this study, solubility of venetoclax was measured in three different biorelevant media (FaSSIFp, FaSSIF and FeSSIF) and compared to solubility values obtained in *ex vivo* porcine intestinal fluids (PIF). The comparisons revealed that using human biorelevant media to estimate *in vivo* solubility in pigs does not appear adequately predictive. The solubilities measured in FaSSIF (5.2 ± 0.1 μg/mL) under predicted the solubility in PIF_{Fasted} (163 ± 82 μg/mL), showing a 30-fold lower solubility. Solubility in FaSSIFp (154 ± 15 μg/mL) was similar to the obtained solubility in PIF_{Fasted} and therefore supports the use of this simulated media in estimating luminal solubility in pigs in the fasted state. As there is currently no available biorelevant medium simulating PIF_{Fed}, *ex vivo*

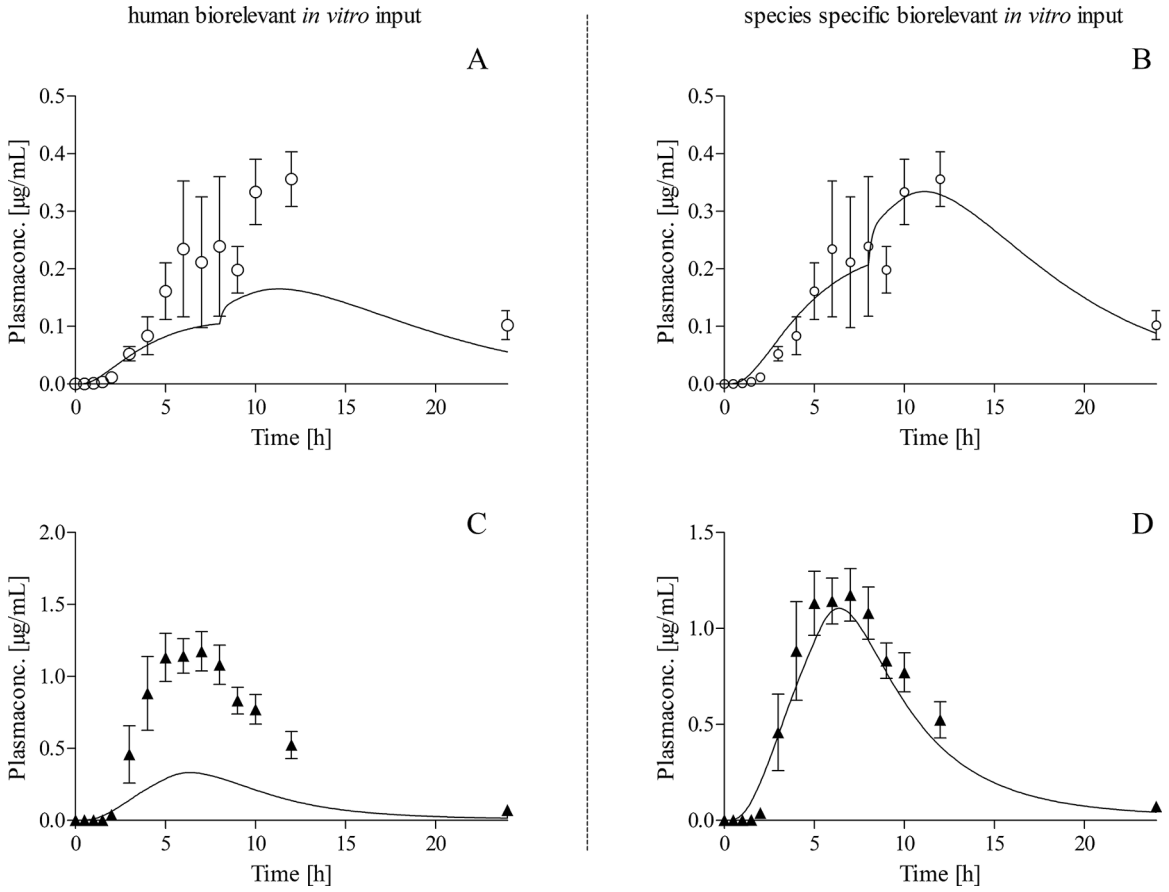

Fig. 6. GastroPlus™ simulations- in silico model of plasma venetoclax concentration vs time profile compared to observed in vivo pig data, A: solid line indicated the predicted fasted profile, based on in vitro FaSSIF data; B: solid line indicated the predicted fasted profile, based on in vitro FaSSIFp data (reproduced from Fig. 4D); C: solid line indicated the predicted fed profile, based on in vitro FeSSIF data; D: solid line indicated the predicted fed profile, based on in vitro FaSSIFp and PIF_{fed} data (reproduced from Fig. 5; (o) indicates the observed fasted state data, (▲) indicated the observed fed state data (n=5, mean ±SEM).

measurements of venetoclax solubility in PIF_{Fed} were used to estimate *in vivo* intestinal solubility in the fed state pigs. An increase in solubility using fed state media was observed (ratio of 5.5 FeSSIF/FaSSIF; 1.8 PIF_{fed}/PIF_{fasted}), which is in line with clinical observation for venetoclax when co-administered with food, reflecting a 4.5 ± 3.2-fold increase of AUC after a high fat meal (753 kcal) and 3.1 ± 2.3–fold increase of AUC after a low fat meal (512 kcal) in contrast to fasted state conditions (Salem et al., 2016).

To date, most of the published studies with PBPK models in preclinical species either utilize human biorelevant media or attempt extrapolations from the human biorelevant media to preclinical species based on the expected changes in bile micelle concentrations (Lignet et al., 2016; Suenderhauf and Parrott, 2013). Therefore, one of the primary aims of this study was to assess the capability of porcine specific simulated intestinal fluids to mimic luminal drug concentrations in combination with a porcine *in silico* model to forecast plasma concentrations *in vivo*. Accurate simulation of animal studies requires the appropriate solubility input, and this study demonstrated the merits of a species specific biorelevant *in vitro* testing for providing more accurate prediction *in vivo* (Fig. 6). The use of solubility estimates from human biorelevant media (FaSSIF and FeSSIF) as inputs to the specific landrace pig *in silico* model, incorporating physiological data for gastric emptying and gastrointestinal fluid volumes in landrace pigs reported by Henze et al. (2019), resulted in a poor correlation with porcine *in vivo* data, in both the fasted and fed state (Fig. 6 A and Fig. 6 C). By using human FaSSIF to predict plasma concentrations in pigs clearly under-predicted the observed drug plasma concentration for venetoclax (predicted $AUC_{0-\infty}$ 2.9 μg*h/mL compared to observed $AUC_{0-\infty}$ 5.5 μg*h/mL (% PE -50)). However, the use of porcine specific *in vitro* data measured in FaSSIFp, improved the predictions (Fig. 6 A versus Fig. 6 B) and confirm the importance of using appropriate input parameters to model preclinical conditions (Fig. 6 B and D).

For predicting fed state conditions, solubility from *ex vivo* PIF_{fed}, combined with dissolution kinetics obtained from FaSSIFp, successfully predicted the food induced increase in absorption of venetoclax. This reaffirms the importance of using biorelevant input parameters, as parameters based on human biorelevant media under predicted the observed fold increase of venetoclax *in vivo* (Fig. 6 C). This approach of using an integrated porcine PBPK model therefore offers a novel approach of mechanistically capturing the food effect of bioenabling formulations in a preclinical setting. While availability of *ex vivo* PIF_{fed} to estimate luminal solubility in the fed state, as employed here, has limitations for more widespread applications as a preclinical tool, approaches to establish a media for simulating fed state conditions in pigs are therefore merited going forward.

Our findings are similar to those studies reported for dogs, where Walsh and co-workers reported improved predictions of plasma concentration profiles in dogs, when using data obtained in canine fasted simulated intestinal fluid. In their study *in vitro* results indicated that canine biorelevant media resulted in a 40-fold higher solubility of the investigational drug ('compound free acid 6') relative to human FaSSIF, which led to significant under-prediction of exposures for the drug when using human FaSSIF as an input parameter(Jones et al., 2006; Walsh et al., 2017). Given that both dogs and pigs are widely used in preclinical screening, it is important to establish species related differences in drug solubility and dissolution characteristics, and the usage of specific biorelevant *in vitro* tools provide a useful approach of addressing this need. This may further provide a useful tool in guiding the selection of an appropriate preclinical model and also offering the advantages of reducing repeated *in vivo* testing in different animal models.

The present mechanistic PBPK models have shown success in prospectively simulating absorption of venetoclax in pigs. It must be noted that species specific gastrointestinal transit times need to be accounted when simulating intestinal absorption, the default minipig ACAT model includes a gastric transit time of 0.70 h. However, based on recent studies (Henze et al., 2018a; Henze et al., 2020; Suenderhauf et al., 2014), gastric emptying is prolonged in pigs, and adjustment to more appropriate estimates can improve the *in silico* model significantly. This is in line with the findings from Suenderhauf and co-workers, the established PBPK model for Göttingen minipigs was refined by using prolonged gastric emptying rate, which lead to more accurate PK predictions of the model compounds (Suenderhauf and Parrott, 2013). The adjusted minipig ACAT™ model used in this study further incorporated pig specific gastrointestinal adjustments and resulted in improved correlation to *in vivo* data, capturing the observed plasma concentration of venetoclax in fasted pigs (Fig. 4 D).

This study further demonstrated the utility of the pig model to predict the food effects of poorly water soluble drugs in humans. A significant increase in absolute bioavailability of 28.7 ± 7.5 % in the fed state compared to 12.9 ± 3.7 % in the fasted state, reflecting a 2.3 ± 0.5-fold increase ($p < 0.05$), showed that venetoclax absorption is a higher under postprandial conditions in pigs. The observed increase in bioavailability of venetoclax in landrace pigs was in line with observation from clinical food effect studies, where the reported food effect of venetoclax in humans was approximately 3.1 ± 2.3 –fold higher after a low fat meal (512 kcal) and 4.5 ± 3.2-fold after a high fat meal (753 kcal) (Salem et al., 2016). Previously we have established a food effect protocol in landrace pigs, and demonstrated, using fenofibrate, a significant 2-fold higher bioavailability in the fed state over the 0- 8 h post dosing period (Henze et al., 2019). This observation was in line with food effect previously reported for fenofibrate in humans. Applying the same porcine food effect protocol for venetoclax, similarly demonstrated a significantly increased bioavailability after administration with the FDA style high fat breakfast to pigs, with an 8.4-fold higher AUC_{fed}/AUC_{fasted} ratio over the 0- 8 h post dosing period. Similar to our previous study, a distinct secondary absorption phase was observed on feeding the pigs after 8 h, and while this reduced the overall AUC_{fed}/AUC_{fasted} ratio to 2.3 ± 0.5, overall the statistical significance was maintained over the complete period of 0- ∞ h.

5. Conclusion

In summary, an important aim of this work was to capture the venetoclax plasma concentration profile under fasted and fed conditions in pigs, by accurately incorporating biorelevant solubility and biorelevant dissolution using a novel pig biorelevant medium. The optimized pig ACAT model was able to mechanistically capture the disposition of venetoclax in pigs, while accounting for species specific dissolution and solubility inputs. This study showed that the applied porcine physiologically based absorption model was able to prospectively simulate the *in vivo* behaviour of venetoclax in landrace pigs, by combining porcine biorelevant *in vitro* and *in silico* tools. Using *in vitro* solubility and dissolution data for venetoclax measured in species specific simulated fluids (FaSSIFp), correctly predict the observed *in vivo* drug levels of venetoclax in fasted pigs. In addition, by combining porcine luminal solubility and porcine biorelevant dissolution data with *in silico* modelling, accurately predicted the observed preclinical food effect *in vivo*. The study further confirmed that the utility of the landrace pig model for predicting food effect in humans, and in this case for a BCS class IV compound. Overall the findings demonstrate the merits of integrating physiologically-informed *in vitro* and *in silico* tools for predicting drug product performance in a preclinical setting, and advance the use of these approaches for optimising clinical drug product design at an earlier stage in development.

CRediT authorship contribution statement

Laura J. Henze: Writing – original draft, Methodology, Conceptualization. **Niklas J. Koehl:** Data curtion, Visualization, Methodology. **Joseph P. O'Shea:** Data curtion, Visualization. **René Holm:** Supervision. **Maria Vertzoni:** Supervision. **Brendan T. Griffin:** Supervision.

Acknowledgments

The authors are part of the PEARRL European Training network, which has received funding from the Horizon 2020 Marie Sklodowska-Curie Innovative Training Networks programme under grant agreement No. 674909.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2021.105840.

References

Arndt, M., Chokshi, H., Tang, K., Parrott, N.J., Reppas, C., Dressman, J.B., 2013. Dissolution media simulating the proximal canine gastrointestinal tract in the fasted state. Eur. J. Pharm. Biopharm. 84, 633–641.

Augustijns, P., Wuyts, B., Hens, B., Annaert, P., Butler, J., Brouwers, J., 2014. A review of drug solubility in human intestinal fluids: implications for the prediction of oral absorption. Eur. J. Pharm. Sci. 57, 322–332.

Bergstrom, C.A., Holm, R., Jorgensen, S.A., Andersson, S.B., Artursson, P., Beato, S., Borde, A., Box, K., Brewster, M., Dressman, J., Feng, K.I., Halbert, G., Kostewicz, E., McAllister, M., Muenster, U., Thinnes, J., Taylor, R., Mullertz, A., 2014. Early pharmaceutical profiling to predict oral drug absorption: current status and unmet needs. Eur. J. Pharm. Sci. 57, 173–199.

Emami Riedmaier,, A., Lindley,, D.J., Hall,, J.A., Castleberry,, S., Slade,, R.T., Stuart,, P., Carr,, R.A., Borchardt,, T.B., Bow,, D.A.J., Nijsen,, M., 2018. Mechanistic physiologically based pharmacokinetic modeling of the dissolution and food effect of a biopharmaceutics classification system IV compound-the venetoclax story. J. Pharm. Sci. 107, 495–502.

FDA, 2002. Food-effect bioavailability and fed bioequivalence studies: guidance for industry. Food-effect bioavailability and fed bioequivalence studies: guidance for industry. http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM126833.pdf.

FDA, 2015. Venclexta (Venetoclax) Clinical pharmacology and biopharmaceutics review (s) application number: 208573Orig1s000. In: review, O.oO.cC.pP. (Ed.), U.S. Department of Health and Human Services. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2016/208573Orig1s000ClinPharmR.pdf.

Henze, L.J., Griffin, B.T., Christiansen, M., Bundgaard, C., Langguth, P., Holm, R., 2018a. Exploring gastric emptying rate in mini-pigs: effect of food type and pre-dosing of metoclopramide. Eur. J. Pharm. Sci.

Henze, L.J., Koehl, N.J., Jansen, R., Holm, R., Vertzoni, M., Whitfield, P., Griffin, B.T., 2020. Development and evaluation of a biorelevant medium simulating porcine gastrointestinal fluids. Eur. J. Pharm. Biopharm.

Henze, L.J., Koehl, N.J., O'Shea, J.P., Holm, R., Vertzoni, M., Griffin, B.T., 2019. Toward the establishment of a standardized pre-clinical porcine model to predict food effects - case studies on fenofibrate and paracetamol. Int. J. Pharm. X 1, 100017.

Henze, L.J., Koehl, N.J., O'Shea, J.P., Kostewicz, E.S., Holm, R., Griffin, B.T., 2018b. The pig as a preclinical model for predicting oral bioavailability and in vivo performance of pharmaceutical oral dosage forms: a PEARRL review. J. Pharm. Pharmacol.

ICH Guideline, 2005. In: Validation of analytical procedures: text and methodology Q2 (R1), International conference on harmonization, Geneva, Switzerland. ICH harmonised tripartite guideline.

Jantratid, E., Janssen, N., Reppas, C., Dressman, J.B., 2008. Dissolution media simulating conditions in the proximal human gastrointestinal tract: an update. Pharm. Res. 25, 1663–1676.

Jones, H.M., Parrott, N., Ohlenbusch, G., Lave, T., 2006. Predicting pharmacokinetic food effects using biorelevant solubility media and physiologically based modelling. Clin. Pharmacokinetics 45, 1213–1226.

Jones, A.K., Freise, K.J., Agarwal, S.K., Humerickhouse, R.A., Wong, S.L., Salem, A.H., 2016. Clinical predictors of venetoclax pharmacokinetics in chronic lymphocytic leukemia and non-Hodgkin's lymphoma patients: a pooled population pharmacokinetic analysis. AAPS J. 18, 1192–1202.

Klein, S., 2010. The use of biorelevant dissolution media to forecast the in vivo performance of a drug. AAPS J. 12, 397–406.

Koehl, N.J., Henze, L.J., Kuentz, M., Holm, R., Griffin, B.T., 2020. Supersaturated lipid-based formulations to enhance the oral bioavailability of venetoclax. Pharmaceutics 12.

Koziolek, M., Alcaro, S., Augustijns, P., Basit, A.W., Grimm, M., Hens, B., Hoad, C.L., Jedamzik, P., Madla, C.M., Maliepaard, M., Marciani, L., Maruca, A., Parrott, N., Pavek, P., Porter, C.J.H., Reppas, C., van Riet-Nales, D., Rubbens, J., Statelova, M., Trevaskis, N.L., Valentova, K., Vertzoni, M., Cepo, D.V., Corsetti, M., 2019. The mechanisms of pharmacokinetic food-drug interactions - A perspective from the UNGAP group. Eur. J. Pharm. Sci. 134, 31–59.

Lignet, F., Sherbetjian, E., Kratochwil, N., Jones, R., Suenderhauf, C., Otteneder, M.B., Singer, T., Parrott, N., 2016. Characterization of pharmacokinetics in the Gottingen Minipig with reference human drugs: an in vitro and in vivo approach. Pharm. Res. 33, 2565–2579.

O'Shea, J.P., Faisal, W., Ruane-O'Hora, T., Devine, K.J., Kostewicz, E.S., O'Driscoll, C. M., Griffin, B.T., 2015. Lipidic dispersion to reduce food dependent oral bioavailability of fenofibrate: in vitro, in vivo and in silico assessments. Eur. J. Pharmaceut. Biopharmaceut. 96, 207–216.

O'Shea, J.P., Holm, R., O'Driscoll, C.M., Griffin, B.T., 2018. Food for thought: formulating away the food effect - a PEARRL review. J. Pharm. Pharmacol.

Pathak, S.M., Ruff, A., Kostewicz, E.S., Patel, N., Turner, D.B., Jamei, M., 2017. Model-based analysis of biopharmaceutic experiments to improve mechanistic oral absorption modeling: an integrated in vitro in vivo extrapolation perspective using ketoconazole as a model drug. Mol. Pharm. 14, 4305–4320.

Salem, A.H., Agarwal, S.K., Dunbar, M., Nuthalapati, S., Chien, D., Freise, K.J., Wong, S. L., 2016. Effect of low- and high-fat meals on the pharmacokinetics of venetoclax, a selective first-in-class BCL-2 inhibitor. J. Clin. Pharmacol. 56, 1355–1361.

Sjogren, E., Abrahamsson, B., Augustijns, P., Becker, D., Bolger, M.B., Brewster, M., Brouwers, J., Flanagan, T., Harwood, M., Heinen, C., Holm, R., Juretschke, H.P., Kubbinga, M., Lindahl, A., Lukacova, V., Munster, U., Neuhoff, S., Nguyen, M.A., van Peer, A., Reppas, C., Hodjegan, A.R., Tannergren, C., Weitschies, W., Wilson, C., Zane, P., Lennernas, H., Langguth, P., 2014. In vivo methods for drug absorption - Comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects. Eur. J. Pharmaceut. Sci. 57, 99–151.

Suenderhauf, C., Parrott, N., 2013. A physiologically based pharmacokinetic model of the Minipig: data compilation and model implementation. Pharm. Res. 30, 1–15.

Suenderhauf, C., Tuffin, G., Lorentsen, H., Grimm, H.P., Flament, C., Parrott, N., 2014. Pharmacokinetics of paracetamol in Gottingen minipigs: in vivo studies and modeling to elucidate physiological determinants of absorption. Pharm. Res. 31, 2696–2707.

Vertzoni, M., Dressman, J., Butler, J., Hempenstall, J., Reppas, C., 2005. Simulation of fasting gastric conditions and its importance for the in vivo dissolution of lipophilic compounds. Eur. J. Pharm. Biopharm. 60, 413–417.

Walsh, P.L., Stellabott, J., Nofsinger, R., Xu, W., Levorse, D., Galipeau, K., Kesisoglou, F., 2017. Comparing dog and human intestinal fluids: implications on solubility and biopharmaceutical risk assessment. AAPS PharmSciTech. 18, 1408–1416.