Simulating intestinal transporter and enzyme activity in a physiologically based pharmacokinetic model for tenofovir disoproxil fumarate

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Synopsis

Tenofovir disoproxil fumarate (TDF), a prodrug of tenofovir, has oral bioavailability (25%) limited by intestinal transport (p-glycoprotein), and intestinal degradation (carboxylesterase). However, the influence of luminal pancreatic enzymes is not fully understood. Physiologically-based pharmacokinetic (PBPK) modelling has utility for estimating drug exposure from in vitro data. This study aimed to develop a PBPK model that included luminal enzyme activity to inform dose reduction strategies. TDF and TFV stability in porcine pancrelipase concentrations was assessed (0, 0.48, 4.8, 48 and 480U/mL lipase; 1mM TDF; 37˚C; 0 – 30 min). Samples were analyzed using mass spectrometry. TDF stability and permeation data allowed calculation of absorption rates within a human PBPK model to predict plasma exposure following six days of once-daily 300 mg TDF dose. Regional absorption of drug was simulated across gut segments. TDF was degraded by pancrelipase (half-life 0.07 and 0.62 hours using 480 and 48 U/mL, respectively). Previous literature C_{max} (335 ng/mL), T_{max} (2.4 hr) and $AUC_{0\text{-}24hr}$ (3045 ng.hr/mL) and C_{24hr} (48.3 ng/mL) were all within 0.5-fold difference of the simulated C_{max} (238 ng/mL), T_{max} (3 hr) and $AUC_{0\text{-}24hr}$ (3036 ng.hr/mL) and C_{24hr} (42.7 ng/mL). Simulated TDF absorption was higher in duodenum and jejunum than ileum ($p<0.05$). These data support that TDF absorption is limited by the action of intestinal lipases. Results suggest that bioavailability may be improved by protection of drug from intestinal transporters and enzymes, for example by co-administration of enzyme inhibiting agents or nanoformulation strategies.

1. Introduction

Human immunodeficiency virus (HIV) is currently an incurable disease which constitutes a serious global health crisis. Oral antiretroviral therapy is the mainstay of current therapy and involves coadminstration of drugs targeting multiple viral targets. Both drug-related adverse reactions and drug resistance have led to the development of newer antiretroviral drugs and drug classes. However, the cost and dosing schedule of treatments are a significant concern in low- and middle-income countires (L&MICs). Several effective antiretroviral drugs are now manufactured as generics and are marketed with significant cost savings to payers (1, 2).

Tenofovir disoproxil fumarate (TDF), a prodrug of tenofovir (TFV), is a cornerstone of first-line treatment in low-income and middle-income countries. TDF is a nucleotide-reverse transcriptase inhibitor (NtRTI), which prevents viral DNA chain lengthening and replication (3) (Figure 1). During preclinical development, TFV demonstrated low oral bioavailability (around 13%) (4) and TDF was developed to improve this by removing charged regions and increasing lipophilicity. However, the oral bioavailability of TDF was only moderately improved and is estimated at around 25% in fasted subjects (5, 6). Substantially improving the bioavailability of TDF would provide significant cost-saving in L&MICs. In order to achieve this, it is important to understand the causes of low TDF bioavailability and to formulate potential targeting strategies.

Using Caco-2 monolayers as a model for the intestinal epitheial surface, the drug efflux transporter ABCB1 has been shown to readily transport TDF (7). As ABCB1 is involved in the elimination of substrates from the enterocytes of the intestine back

into the luminal space, it is hypothesised that inhibitors of ABCB1 may improve TDF oral absorption. However, this ABCB1 inhibitory effect was not observed in situ using perfusion studies in rats (8). The significance of this finding is not clear due to the significantly faster TDF degradation in rat duodenal and ileal enterocytes compared to corresponding human enterocytes (9).

Previous studies have investigated the impact of co-administered HIV protease inhibitors on TFV pharmacokinetics (7). Ritonavir-boosted protease inhibitors atazanavir, lopinavir, darunavir and saquinavir all showed a modest increase in TFV exposure when co-administered with TDF. Protease inhibitors show varying degrees of ABCB1 inhibition *in vitro*. Therefore, the same study also investigated the impact of these drugs on ABCB1-mediated transport of TDF. Darunavir, which in patients led to a 22% increase in TFV exposure, had no impact on TDF transport *in vitro*. In the case of the interactions observed with protease inhibitors and possibly other drugs, additional bioavailability-related mechanisms are likely to be involved alongside ABCB1.

TDF is rapidly metabolised to TFV in the presence of rat intestinal microsomes (8). It was hypothesised that carboxylesterase was responsible for this metabolism, and further investigations showed improved drug stability in the presence of certain natural fruit extracts and pharmacological esters. Protease inhibitors have shown varying degrees of carboxylesterase inhibition, and this enzyme may therefore be involved in interactions between TDF and these drugs.

Pancreatic enzymes, including lipase, amylase and trypsin, are released into the duodenum and are involved in the breakdown of ingested food in preparation for nutrient absorption. Pancreatic lipase is known to hydrolyse ester bonds (10), but the ability of lipase to metabolise TDF by ester-linked chain removal has not been previously investigated.

The initial aim of this study was to determine *in vitro* the extent of TDF metabolism by pancreatic lipase. This data was combined with previous information on TDF ABCB1-mediated transport and carboxylesterase metabolism to create a PBPK model with informed absorption mechanisms involving all these processes. The model generated was then used to estimate the relative importance of these factors in TDF absorption, providing a platform to postulate possible dose reduction strategies.

2. Results

2.1 Stability of TDF in pancrelipase

The breakdown of TDF was determined at various concentrations of lipase. The half-life of TDF was determined as 4.0 minutes in 480 U/mL lipase and 37.5 minutes in 48 U/mL lipase. TDF was stable in 4.8 U/mL lipase and below, therefore half-life was not determined at these concentrations. Data was used to generate an equation, given below, relating lipase concentration to TDF stability, which was used to inform the PBPK model.

$$TDF_{1/2} = -0.0013 * [Lipase] + 0.6863$$

Where $TDF_{1/2}$ is the half life of TDF (hr); [Lipase] is the concentration of lipase in the intestine (units/mL). To confirm the role of lipase in TDF degradation, selective inhibition of pancreatic enzymes were performed and the influence on TDF half life was evauated. Co-incubation of TDF with lipase inhibitor orlistat resulted in a 33% increase in TDF half life (16.0 min) compared to inhibitor-free incubation (12.0 min, $p = 0.02$), whereas no alteration in TDF half life was observed with co-incubation with amylase inhibitor acarbose (12.6 min, $p = 0.66$) or trypsin inhibitor type 1-S from glycine max (soybean) (12.7 min, $p = 0.23$).

2.2 Model verification

The 300 mg once-daily steady state TFV pharmacokinetic study by Barditch-Crovo et al showed variability in median pharmacokinetic parameters C_{max}, T_{max}, $AUC_{0\text{-}24hr}$

assessed between days 8, 15 and 35 days of the study (6). Therefore, a mean and standard deviation between these assessment days was calculated and used for comparison with simulated data. The clinical C_{max} (335 ng mL^{-1}), T_{max} (2.4 hr) and $AUC_{0\text{-}24hr}$ (3045 ng.hr mL^{-1}) were all within 0.5-fold difference of the simulated C_{max} (238 ng mL^{-1}, 0.41-fold higher), T_{max} (3 hr, 0.2-fold lower) and $AUC_{0\text{-}24hr}$ (3036 ng.hr mL^{-1}, 0-fold difference). The observed median TFV C_{24hr} following seven days of once-daily 300 mg oral dosing was 48.3 ng mL^{-1}, which was within the acceptable range of the median simulated TDF C_{24hr} of 42.7 ng mL^{-1} (Figure 2A). The terminal plasma half life of TFV was determined as 11.7 hours and 8.5 hours from the median clinical and simulated pharmacokinetic data, respectively, following seven days of 300 mg once-daily oral dosing. The simulated mean bioavailability of 300 mg of orally dosed TDF was 21.2 ± 3.3 % (range 13.1-27.6 %). This estimation of oral bioavailability slightly underpredicted the 25% value estimated to be bioavailable from clinical studies. To validate the robustness of the model further, simulations were performed as described above but using alternative TDF oral dose sizes (75 mg, 150 mg and 600 mg) and plasma concentration curves compared with previous clinical data (6). For the 75 mg TDF dose, the clinical C_{max} (53 ng mL^{-1}) and T_{max} (2.5 hr) were within 0.5-fold difference of the simulated C_{max} (51 ng mL^{-1}, 0.03-fold higher) and T_{max} (3 hr, 0.2-fold lower) (Figure 2B). The $AUC_{0\text{-}24hr}$ and C_{24hr} for the 75 mg dose could not be determined from the clinical data as TFV was undetectable at the 0 and 24 hour time points on the plasma concentration curve. Therefore, clinical $AUC_{1\text{-}12hr}$ (480 ng.hr mL^{-1}) and C_{12hr} (34 ng mL^{-1}) were compared to simulated $AUC_{1\text{-}12hr}$ (488 ng.hr mL^{-1}, 0.02-fold lower) and C_{12hr} (28 ng mL^{-1}, 0.21-fold higher) (Figure 2B). For the 150 mg TDF dose, the clinical C_{max} (135 ng mL^{-1}), T_{max} (2.5 hr), $AUC_{0\text{-}24hr}$ (1581 ng.hr mL^{-1}) and C_{24hr} (29 ng mL^{-1}) were all within 0.5-fold difference of the

simulated C_{max} (112 ng mL^{-1}, 0.21-fold higher), T_{max} (2 hr, 0.25-fold higher), AUC_{0-24hr} (1581 ng.hr mL^{-1}, 0-fold difference) and C_{24hr} (23 ng mL^{-1}, 0.26-fold higher) (Figure 2C). For the 600 mg TDF dose, the clinical C_{max} (618 ng mL^{-1}), T_{max} (2.5 hr), AUC_{0-24hr} (6166 ng.hr mL^{-1}) and C_{24hr} (111 ng mL^{-1}) were all within 0.5-fold difference of the simulated C_{max} (574 ng mL^{-1}, 0.08-fold higher), T_{max} (2 hr, 0.25-fold higher), AUC_{0-24hr} (7547 ng.hr mL^{-1}, 0.18-fold lower) and C_{24hr} 106 ng mL^{-1}, 0.05-fold higher) (Figure 2D).

2.3 Simulations of TDF and TFV fractional absorption

The regional intestinal absorption of TDF and TFV was simulated over 24 hours in 100 virtual subjects following a single 300 mg oral dose of TDF (Figure 3). The majority of drug entering the systemic circulation was predicted to be accounted for by absorption of TDF directly, predominantly via the duodenum (11.7 mg) and section 1 of the jejunum (11.6 mg). Compared to absorption of TDF, overall absorption of TFV was predicted to be minor and varied from 0.15 mg in the duodenum to 1.19 mg in section 2 of the jejunum.

2.4 Simulations of tenofovir plasma concentrations following inhibition of intestinal ABCB1, CES and luminal lipase

Data was found to be non-normally distributed so a Mann Whitney U test was used to determine significance. The median tenofovir AUC_{0-24hr} (5^{th}-95^{th} centile) of the control group (3036 (1752-4762) ng.hr mL^{-1}) was significantly less than other groups following inhibition of ABCB1 (4480 (2538-7228) ng.hr mL^{-1}), $p<0.01$), ABCB1 +

carboxylesterase (6018 (3514-10976) ng.hr mL^{-1}), p<0.01), lipase (12873 (9020-20827) ng.hr mL^{-1}), p<0.01), ABCB1 + lipase (17322 (12752-26226) ng.hr mL^{-1}), p<0.01), and ABCB1 + carboxylesterase + lipase (19250 (14215-29208) ng.hr mL^{-1}), p<0.01) (Table 2).

2.5 Simulations of dose reduction strategies

Further simulations were performed where TDF dose was reduced in groups with inhibited factors, with the aim of achieving a similar (within 10% difference) median tenofovir $AUC_{0\text{-}24hr}$ as was observed in the control group. Comparable tenofovir exposure was observed in all simulated groups following specific dose reductions in each case (Table 3). The median $AUC_{0\text{-}24hr}$ (5^{th}-95^{th} centile) following inhibition of ABCB1 (200 mg dose, 2897 (1678-4603) ng.hr mL^{-1}), ABCB1 + carboxylesterase (150 mg dose, 3002 (1900-4536) ng.hr mL^{-1}), lipase (90 mg dose, 3337 (2245-5341) ng.hr mL^{-1}), ABCB1 + lipase (60 mg dose, 3279 (2366-4950) ng.hr mL^{-1}), and ABCB1 + carboxylesterase + lipase (50 mg dose, 3133 (2286-4751) ng.hr mL^{-1}) were all within 10% difference of the control group (3036 (1752-4762) ng.hr mL^{-1}).

3. Discussion

In this study we have established that TDF is unstable in the presence of physiologically relevant concentrations of pancrelipase. The most likely mechanism of this degradation is ester bond cleavage that first results in the TFV monoester and finally in TFV. As this process occurs in the luminal fluid prior to drug absorption, it is hypothesised that luminal lipase activity influences TDF bioavailability in humans. To investigate this, the impact of luminal lipase was included in the PBPK model to estimate TFV plasma concentrations. Simulations suggest that the included factors contribute to the TFV exposure seen in subjects, with luminal lipase having a significant impact. In addition to results generated in this study, there is supporting evidence that lipase may be a relevant factor in determining TFV bioavailability. Protease inhibitors are able to inhibit lipase activity *in vitro* (11) and this process may be involved in the interactions seen between TDF and these drugs (possibly in addition to its known inhibition of transporters). Additionally, TDF exposure was increased 40% in human subjects when taken with a high-fat meal whereas no comparable effect was seen with a low-fat meal (3). Although it is common to see improved biovavailability of highly lipophilic and insoluble drugs when taken with a fatty meal, TDF is reasonably soluble in intestinal fluid. Alternatively, it can be hypothesised that the increased fat in the intestinal fluid limits availability of lipase active sites for TDF metabolism. However, this competition has not been assessed and requires further empirical confirmation.

The authors have successfully employed PBPK modelling to include a variety of factors that influence TDF absorption. However, due to existing knowledge gaps and

the complexities of biological processes involved in TFV pharmacokinetics, there are limitations to this approach and it is important that these limitations are addressed. The simulations were undertaken assuming a population of fasted subjects, and the influence of ingested food and fats was not considered. This was due to incomplete information on the effects of food and fats on intestinal lipase activity and the influence of this on TDF stability. It was assumed that lipase was only active in the small intestine compartments and that the level of activity did not vary between these compartments in individual subjects. Per amount of enzyme, the activity level of the lipase was assumed to be similar in both an *in vitro* and *in vivo* environment, and in the *in vitro* experiments the authors used FaSSIF to replicate the environment in the fasted-state luminal fluid. Additionally, the use of porcine pancrelipase was chosen by the authors due to the well characterised enzymatic activity of the product, where precise units of all enzymes (ie activity of lipase, amylase and trypsin) per weight of substance was known, whereas human pancreatic fluids available to the authors did not provide the required information needed for utilisation in the PBPK model. The distribution levels and activity levels of carboxylesterase protein in different sections of the intestine is unknown; therefore it was assumed that the Caco-2 intestinal model was a suitable surrogate system.

Reformulation of TDF offers a strategy to improve bioavailability. There are cases where multiple formulations of an antiretroviral are available, often for specific scenarios such as in paediatric treatment. Comparison studies have shown that formulation composition can significantly influence antiretroviral pharmacokinetics (1). Extended release formulations have proven beneficial in many diseases, and

may have the potential to protect TDF from luminal enzymes. Inhibition of ABCB1 or carboxylesterase alone is unlikely to have a dramatic effect on TFV bioavailability, but a more holistic approach to inhibit multiple proteins (including lipase) may be more successful. This is somewhat supported by the modest increase in TFV exposure observed on coadminstration with boosted protease inhibitors, and a more target-driven approach may achieve greater increases. Emerging nanotechnologies may also provide bespoke opportunities to encapsulate and protect TDF from degradation until absorption is complete (12).

4. Materials and Methods

4.1 Determination of TDF stability in pancrelipase

The stability of 1 mM TDF was assessed in triplicate in a range of lipase concentrations (0, 0.48, 4.8, 48 and 480 U/mL) using porcine pancrelipase. The medium used to perform experiments was Fasted Simulated Small Intestinal Fluid (FaSSIF; 3 mM sodium taurocholate, 0.2 mM lecithin, 34.8 mM sodium hydroxide, 68.62 mM sodium chloride, maleic acid 19.12 mM, deionized water 1L, hydrogen chloride added dropwise to achieve pH 6.5) and TDF concentrations were assessed at 0, 5, 10, 15, 20 and 30 minutes. Parallel experiements were performed to identify the specific enzymes involved in TDF degradation, where saturating concentrations of inhibitors of lipase (100 µg/mL orlistat), amylase (1 mg/mL acarbose) and trypsin (1 mg/mL Type 1-S trypsin inhibitor from soybean) (13) were co-incubated with 20 µg/mL TDF and alterations in TDF half life were determined in the presence of a mix of porcine pancreatic enzymes (100 U/mL lipase, 540 U/mL amylase and 340 U/mL trypsin). All experiements were performed at 37ºC and samples were processed and

analysed at Scynexis (Durham, NC, USA) using LC-MS/MS. The column used was a Synergi Polar RP 2.0x150mm 4um (Phenomenex) kept at 60˚C, mobile phase A consisted of 96%/3%/1% water/acetonitrile/acetic acid and mobile phase B consisted of 3%/96%/1% water/acetonitrile/acetic acid. Flow rate was 600 µL/min and consisted of 100% A between 0 to 1 minutes, 2% A 98% B at 2 minutes, 2% A 98% B at 3 minutes, 100% A at 3.1 minutes, 100% A at 4 minutes. TDF was detected in positive mode, Q1 mass was 520.1 and Q3 mass was 270 using collision energy 34. Labetalol was used as internal standard.

4.2 Model construction

The PBPK model was created using SimBiology version 3.3, a product of Matlab v.8.2 (MathWorks, Natick, MA, USA, 2013). The aim of the PBPK model was to simulate the steady-state pharmacokinetics of TFV in humans following six days of once-daily 300 mg TDF administration. In particular, the aspects potentially relevant to TDF absorption (solubility, lipase activity, carboxylesterase activity, ABCB1 activity) were included to simulate the relative importance of each factor.

4.3 System parameters

The basic structure of the PBPK model is based on previously published model created by the authors (14). Demographic factors of virtual male subjects between the ages of 18 and 60 (height, weight, body mass index, body surface area) were taken from published literature and used in allometric equations to calculate individual organ volumes (15). The volume and rate of blood circulation in each

simulated subject was calculated as previously described (16). The model was created with the following assumptions: 1) tissue compartments were treated as well-stirred compartments with instant distribution of drug; 2) drug was not absorbed from the stomach compartment; 3) the rate of drug absorption from the caecum and colon was reduced to 10-fold less than would be observed in the small intestine under the same conditions; 3) the model is blood-flow-limited. The physiological factors relevant for drug absorption in the intestinal compartments are based on the Advanced Compartmental Absorption and Transit (ACAT) model and are given in Table 1 (17).

4.4 Drug parameters

4.4.1 Solubility of TDF in the luminal fluid

In order to account for potential solubility-induced absorption limitations, the solubility of TDF was measured by Corealis Pharma (Quebec, Canada) in a physiologically-relevant range of buffered pH solutions. Solubility of TDF was high at 9300, 4800 and 6200 mg/L in buffered solutions of pH 2, 4.5 and 8, respectively. These results were then used to derive a quadratic equation, given below, to calculate the pH-dependent limitations to TDF luminal solubility (mg/L) in the PBPK model intestinal compartments.

$$TDFs = (366.67 * [IpH]^2) – (4183.33 * IpH) + 16200$$

317 Where TDFs is the maximum possible solubility of TDF (mg/L); IpH is the pH of the
318 intestinal segment fluid.

319

320 Only soluble TDF, which was continually determined throughout simulations in each
321 intestinal segment, was available for absorption in the PBPK absorption model.

322

323 **4.4.2 Stability of TDF in the luminal fluid**

324 Intestinal lipase concentrations were acquired from literature and included in the
325 small intestine segments of the PBPK model (duodenum, jejunum and ileum) (18).
326 Each simulated fasted subject was given a physiologically-relevant concentration of
327 luminal lipase which was randomly assigned within the ranges obtained from
328 published literature of between 100 and 400 units/mL. Using the *in vitro* metabolism
329 data generated in this study, an equation was then developed establishing the
330 relationship between lipase concentration and drug half life, as given in the results
331 section. The lipase-dependent rate of elimination was then determined for each
332 simulated subject and the degraded TDF was assumed to be converted to TFV,
333 which is either absorbed or passes along and out of the intestine, as detailed below.

334

335 **4.4.3 Absorption of TDF and TFV**

336 TDF permeation through a Caco-2 monolayer has been previously investigated and
337 the apparent permeability (P_{app}) was found to be drug-concentration-dependent (7).
338 The authors hypothesised that this was the result of active transport saturation

(specifically saturation of ABCB1) when higher TDF concentrations were added to the receiver compartment. To inform the current model of this scenario, this relationship between TDF concentrations and P_{app} was continuously re-determined in each intestinal segment using a polynomial equation, given below, derived from a previous study (7).

$$TDF_{Papp} = -1.9*10^{-26} * [TDF_{conc}]^6 + 3.3*10^{-22} * [TDF_{conc}]^5 – 2.2*10^{-18} * [TDF_{conc}]^4 + 7.5*10^{-15} * [TDF_{conc}]^3 – 1.3*10^{-11} * [TDF_{conc}]^2 + 1.1*10^{-8} * [TDF_{conc}] + 1.6*10^{-7}$$

Where TDF_{Papp} is the estimated TDF P_{app} value at a specific concentration of TDF; TDF_{conc} is the concentration of TDF.

Using previously established equations, P_{app} was used to generate the rate of drug absorption in the model (19, 20). Intestinal absorption of TFV, the breakdown product of TDF occurring via luminal lipase, was included. The rate of intestinal TFV absorption was determined by scaling the P_{app} value of 0.41 cm x 10^{-6} s^{-1} determined previously in MDCK monolayers (21) (22).

4.4.4 Distribution of TFV

The volume of distribution of TFV was simulated considering the volume of distribution of 0.813 L/kg described in population pharmacokinetic studies (23) and tissue distribution was determined using previously published equations (24, 25).

4.4.5 Clearance of TFV

TFV is predominantly eliminated from the body unchanged via the kidneys. In order to account for this loss, clearance of TFV was included in the model. The multiple physiological factors involved in the elimination of TFV, such as the effect of drug transporters and tubular reabsorption, have not been fully characterised, making a mechanistic prediction of TFV renal elimination difficult. Therefore, a TFV total clearance rate of 0.066 L/hr/kg was derived from a previous population pharmacokinetic study and was included in our model (6).

4.5 Model verification

To verify the model, pharmacokinetic data from simulations were compared to clinical data. Following six days of once-daily dosing of 300 mg TDF in 100 simulated subjects, median TFV C_{max}, T_{max}, $AUC_{0\text{-}24hr}$ and C_{24hr} were calculated and contrasted to steady state pharmacokinetics observed in real subjects, taken from a randomized, double-blind, placebo-controlled, escalating-dose study of four doses (75, 150, 300, and 600 mg given once daily) with between eight and nine subjects in each group (6). Additionally, the terminal plasma half life of TDF was estimates from simulated concentration plots and compared to half life generated from clinical data. The bioavailability of orally administered TDF is estimated at around 25 % of the total dose (26) and the bioavailability of 300 mg orally administered TDF was determined from our simulations (mean ± standard deviation, with minimum and maximum range) as a comparison to further validate the model. As a pre-determined measure of success for the model validation, a difference of 0.5-fold or less between clinical and predicted pharmacokinetic parameters was deemed acceptably accurate (27) (28). Pharmacokinetic parameters were determined by non-compartmental analysis using PK Solutions 2.0 (Summit Research Services, UK).

To validate the robustness of the model further, simulations were performed as described above but using alternative TDF oral dose sizes (75 mg, 150 mg, 600 mg). Pharmacokinetic parameters (median TFV C_{max}, T_{max}, $AUC_{0\text{-}24hr}$ and C_{24hr}) were generated from these simulations and were compared to available clinical pharmacokinetic data where these dose sizes were utilised (6).

4.6 Assessment of regional absorption of TDF and TFV

The regional absorption of TDF and TFV was simulated in 100 virtual subjects following a single 300 mg oral dose of TDF. Mean absorption amounts (mg) with standard deviations were determined 24 hours post-dose in duodenum, jejunum section 1 (j1), jejunum section 2 (j2), ileum section 1 (i1), ileum section 2 (i2), ileum section 3 (i3), caecum and colon (Table 1).

4.7 Prediction of TFV pharmacokinetics following inhibition of factors involved in absorption

In order to determine the influence of intestinal ABCB1, CES and lipase on tenofovir exposure, each factor was individually (with the exception of CES) and in combination removed from simulations and the pharmacokinetics of TFV determined for each combination. In the case where lipase activity in the model was eliminated, simulations were performed where intestinal $TDF_{1/2}$ = 0 minutes. In the case where ABCB1 activity was inhibited, simulations were performed using a TDF P_{app} value of 3.6 cm x 10^{-6} s^{-1} taken from a previous study (7). In the case where ABCB1 and CES activities in the model were inhibited, simulations were performed using a TDF P_{app} value of 9.41 cm x 10^{-6} s^{-1} taken from a previous Caco-2 permeation study where inhibition of ABCB1 and CES activity was achieved using TPGS (ABCB1 inhibitor) and 1 mM propylparaben (CES inhibitor) (7) (21). In each group, mean tenofovir C_{max}, T_{max}, $AUC_{0\text{-}24hr}$ and C_{24hr} were calculated following six days of once-daily dosing of 300mg TDF in 100 simulated subjects.

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Transparency declaration

None to declare.

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Tables

Table 1. Physiological factors relevant for simulating the oral absorption of TFV disoproxil and TFV in the PBPK model.

Segment	pH	Volume (mL)	Radius (cm)	Transit time (hr)	Absorption scaling
Stomach	1.3	46.56	N/A	0.25	x 0
Duodenum	6	41.56	1.53	0.26	x 1
Jejunum 1	6.2	154.2	1.45	0.93	x 1
Jejunum 2	6.4	122.3	1.29	0.74	x 1
Ileum 1	6.6	94.29	1.13	0.58	x 1
Ileum 2	6.9	70.53	0.98	0.42	x 1
Ileum 3	7.4	49.83	0.82	0.29	x 1
Caecum	6.4	47.49	3.39	4.19	x 1
Colon	6.8	50.33	2.41	12.57	x 0.1

Table 2. Simulated median pharmacokinetic parameters of tenofovir following inhibition of factors involved in drug absorption of TDF. Parameters were determined following 6 days once-daily oral dosing of 300 mg TDF in 100 healthy male subjects.

Inhibited factor	C_{max} (ng mL^{-1})	Tmax (hr)	$AUC_{0\text{-}24hr}$ (ng.hr mL^{-1})	C_{24hr} (ng mL^{-1})
None (control)	238	3	3036	43
ABCB1	377	2	4480	51
ABCB1 + CES	538	2	6018	68
Lipase	1013	3	12873	199
ABCB1 + lipase	1409	3	17322	235
ABCB1 + CES + lipase	1642	3	19250	228

Table 3. Dose reduction strategies following inhibition of factors involved in drug absorption of TDF. Parameters were determined following 6 days once-daily oral dosing of TDF in 100 healthy male subjects.

Inhibited factor	Dose size (mg)	$AUC_{0\text{-}24hr}$ (ng.hr mL^{-1})	% difference from control
None (control)	300	3036	n/a
ABCB1	200	2897	-5%
ABCB1 + CES	150	3002	-1%
Lipase	90	3337	+10%
ABCB1 + lipase	60	3279	+8%
ABCB1 + CES + lipase	50	3133	+3%

Figure legends

Figure 1. The process of converting the prodrug TDF to TFV and ultimately to the active substance TFV diphosphate.

Figure 2. Validation of the physiologically based pharmacokinetic model strategy against clinical data of TDF once-daily, day 7 profiles for dose size of 300 mg (Figure 2A), 75 mg (Figure 2B), 150 mg (Figure 2C) and 600 mg (Figure 2D) (6).

Figure 3. The amount of TDF and tenfovir absorbed via each intestinal segment following a single 300 mg oral dose of TDF (mg ± SD, 24 hours post-dose). J1 = jejunum first section; J2 = jejunum section 2; I1 = ileum section 1; I2 = ileum section 2; I3 = ileum section 3.