Published in final edited form as:

Clin Pharmacokinet. 2015 June ; 54(6): 639–650. doi:10.1007/s40262-014-0227-1.

Physiologically-Based Pharmacokinetic Modelling to Inform Development of Intramuscular Long Acting Nanoformulations for HIV

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Abstract

Background and Objectives—Antiretrovirals (ARVs) are currently used for the treatment and prevention of HIV infection. Poor adherence and low tolerability of some existing oral formulations can hinder their efficacy. Long-acting (LA) injectable nanoformulations could help address these complications by simplifying ARV administration. The aim of this study is to inform the optimisation of intramuscular LA formulations for eight ARVs through physiologically-based pharmacokinetic (PBPK) modelling.

Methods—A whole-body PBPK model was constructed using mathematical descriptions of molecular, physiological and anatomical processes defining pharmacokinetics. These models were validated against available clinical data and subsequently used to predict the pharmacokinetics of injectable LA formulations

Results—The predictions suggest that monthly intramuscular injections are possible for dolutegravir, efavirenz, emtricitabine, raltegravir, rilpivirine and tenofovir provided that technological challenges to control release rate can be addressed.

Conclusions—These data may help inform the target product profiles for LA ARV reformulation strategies.

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Conflict of Interest:

Andrew Owen has received research funding from Merck, Pfizer and AstraZeneca, consultancy from Merck and Norgine, and is a co-inventor of patents relating to HIV nanomedicines. Marco Siccardi has received research funding from ViiV and Janssen. David J Back is a board member in Abbvie, Boehringer Ingelheim, Gilead, Janssen, Merck and ViiV. He receives consulting or advisor fees from Abbvie, Boehringer Ingelheim, Gilead, Janssen, Merck and ViiV. He also received research funding from Abbvie, Boehringer Ingelheim, BMS, Gilead, Janssen, Merck and ViiV. Charles Flexner received consulting or advisor fees from Abbvie, Boehringer Ingelheim, Bristol Myers-Squibb, Gilead and GlaxoSmithKline, Merck and ViiV. Rajith KR Rajoli, Steve Rannard and Caren Freel Meyers have no conflicts of interest to declare.

1 Introduction

The human immunodeficiency virus (HIV) is a global pandemic affecting 75 million people till date, with 2.3 million new cases in 2012 [1]. Modern antiretroviral (ARV) therapy is based on the targeting of specific stages of the viral replication, with optimal suppression obtained through the use of two or more ARV [2] classes in combination. ARVs can also be used for pre-exposure prophylaxis (PrEP) strategies, preventing the transmission of HIV to un-infected individuals who are at high risk of acquiring the infection [3].

Currently available oral formulations necessitate lifelong, daily dosing and subjects often encounter pill fatigue as a consequence. This has the potential of producing suboptimal adherence, which has emerged as one of the main causes of therapeutic failure and low rates of PrEP protection [4]. The introduction of long-acting (LA) formulations could revolutionise treatment and PrEP by enabling administration once-monthly or even less frequently. This can simplify ARV administration and improve adherence [5]. LA formulations are most commonly administered parenterally, decreasing inter- and intra-patient variability in drug exposure. Additional potential advantages include a decrease in overall drug consumption and cost of treatment [6]. LA injectable contraceptives and antipsychotics are widely used and have excellent efficacy and tolerability compared to oral routes of administration [4].

Recently, injectable LA formulations of the ARVs rilpivirine and S/GSK1265744/cabotegravir have been developed with the potential for once monthly dosing, or less frequently [4]. The development of the first injectable LA ARV formulations has led to considerable interest amongst patients; a recent study found that 84% of surveyed patients would definitely or probably try a once-monthly injectable LA ARV [7]. The LA formulations are based on solid drug nanoparticles (SDNs) which release the drug from the depot over a protracted period of time. The optimisation of SDNs for LA administration is complicated by several pharmacokinetic factors and has to take into account the optimal dose and drug release into the circulation to achieve therapeutic or prophylactic plasma concentrations for the entire dosing interval.

The pharmacokinetics of a drug are determined by processes that can be simulated through physiologically-based pharmacokinetic (PBPK) models. PBPK models are based on the mathematical description of anatomical, physiological and molecular processes defining drug distribution, integrating information on drug characteristics and patient-specific factors [8]. This modelling approach can be applied to the development and optimisation of novel formulations to identify optimal pharmacokinetic characteristics [8]. PBPK models have been used extensively in different disease areas for the prediction of pharmacokinetics for novel drugs, optimisation of existing therapies and simulation of different clinical scenarios. Specifically, PBPK can be used to predict pharmacokinetics as a function of formulation and route of administration [9]. These predictions can then inform the design of LA formulations, identifying optimal characteristics in terms of dose and depot release rate. For ARVs, this modelling approach has been applied to identify factors influencing the pharmacokinetics of novel formulations [10, 11] the effect of genetics on drug distribution [11], pharmacokinetics in special populations [12] and drug-drug interactions [13].

The aim of this study was to simulate the pharmacokinetics of ARVs after administration of virtual intramuscular LA formulations using PBPK modelling. The PBPK models were first validated against clinical data for oral counterparts, to assess the accuracy of the simulation. Theoretical target dose and release rate combinations for once weekly and once monthly intramuscular administration were then identified.

2 Methods

Virtual patients between the ages of 18 to 60 years were generated. All organs and tissues were represented as individual compartments. The model was based on the assumptions: 1) well stirred compartments with instant distribution of the drug; 2) no absorption of the drug from the colon; and 3) the model is blood flow limited. The PBPK model was designed using Simbiology v.4.3.1, a product of Matlab v.8.2 (MathWorks, Natick, MA, USA 2013). The PBPK approach has been developed for key ARVs (two nucleoside reverse-transcriptase inhibitors (NRTIs), tenofovir and emtricitabine; three non-nucleoside reverse-transcriptase inhibitors (NNRTIs), efavirenz, rilpivirine and etravirine; two integrase inhibitors (IIs), raltegravir and dolutegravir and an unboosted protease inhibitor (PI), atazanavir.

2.1 Anatomy

The age, body mass index, body surface area, height and weight of the individual were defined as previously described [14]. These initial values were used for the calculation of organ and tissue volumes through allometric equations [14]. The blood circulation was represented by considering the cardiac output and regional blood flows as previously described [14].

2.2 Intestinal absorption

A compartmental absorption and transit model has been integrated to simulate for oral drugs as previously described [15]. Absorption rate was evaluated using the apparent permeability obtained from Caco-2 cells or the polar surface area (PSA) and hydrogen bond donor (HBD) values as previously described [16].

2.3 Intestinal metabolism

The clearance of drugs in the gut (CL_{gut}) was calculated considering the *in vitro* intrinsic clearance (CL_{int}) and the abundance of cytochrome P450 family 3, subfamily A - CYP3A in the intestinal tissue (Ab_{CYP3A}) [16, 17]:

$$CL_{gut} \text{ (L/h)} = \frac{CL_{int} \times Ab_{CYP3A} \times 1000 \times 60}{1000000} \quad (1)$$

The fraction of the drug transiting to the liver after metabolism in the gut (F_g) was computed using the following equation:

$$F_g = \frac{Q_{gut}}{Q_{gut} + f_{u,gut} \times CL_{gut}} \quad (2)$$

where Q_{gut} represents the blood flow to the gut and $f_{u,\text{gut}}$ is the fraction unbound in the gut.

2.4 First pass metabolism

The total intrinsic clearance (TCL_{int}) of an enzyme was calculated as:

$$TCL_{\text{int}} = \frac{CL_{\text{int}} \times \text{Abundance} \times \text{Liver weight} \times MPPGL \times 1000 \times 60}{1000000} \quad (3)$$

where Abundance is the amount of enzyme present in a milligram of microsomal protein and MPPGL is amount of microsomal protein per gram of liver [18]:

$$MPPGL = 10^{1.407 + 0.0158 \times \text{Age} - 0.00038 \times \text{Age}^2 + 0.0000024 \times \text{Age}^3} \quad (4)$$

Then the sum of intrinsic clearances of all the enzymes that are metabolising the drug in the liver gives the total apparent clearance (CL_{app}):

$$CL_{\text{app}} = \sum_{n=1}^n TCL_{\text{int}} \quad (5)$$

The systemic clearance (CL) is calculated from the blood and not the liver and is given by the equation:

$$CL = \frac{Q_{\text{hv}} \times f_u \times CL_{\text{app}}}{Q_{\text{hv}} + CL_{\text{app}} \times f_u} \quad (6)$$

Where Q_{hv} is the hepatic flow rate and f_u is the fraction unbound in plasma.

The fraction of the active drug that is available after first pass hepatic metabolism (F_h) is given as:

$$F_h = 1 - CL/Q_{\text{hv}} \quad (7)$$

2.5 Systemic distribution

The volume of distribution and systemic circulation was calculated using previously published equations [19, 20].

2.6 Intramuscular depot compartment

A compartment was created to virtually represent the depot with a maximum volume of 6 mL since it is the maximum volume of injection that has previously been used for intramuscular administration, although lower volumes are usually needed for optimal tolerability [21]. The blood flow to this area was calculated accordingly. The release of the drug from the depot to the blood capillaries present in the muscle was given as [9]:

$$\frac{d}{dt}A = k \times A \quad (8)$$

where A is the amount of drug released from the depot and k is the first-order release rate. A variability of $\pm 10\%$ for the release rate values was considered.

Also the highest single dose that has been tested clinically is 1500 mg with Cefuroxime [21]. Therefore, any strategy for LA ARV development needs to address depot volume and dose in order to successfully translate.

2.7 Model validation

The physicochemical properties of ARVs used to build the PBPK models are represented in table 1. The models were primarily validated against clinical data at steady state for oral formulations of the ARVs (table 2). The model was also verified against an existing long-acting formulation of rilpivirine [6].

2.8 Prediction of pharmacokinetics following intramuscular injection

In the prediction of intramuscular pharmacokinetics, the dose of the ARV and release rate were optimised such that the mean trough concentration (C_{trough}) was above the IC_{90} , IC_{95} for wild type HIV virus or protein-binding corrected inhibitory concentration ($PBIC_{95}$) after 7- or 30-days after injection. A dose cut-off of 1500 mg was considered for intramuscular administration.

2.9 Prediction of intracellular concentrations

The active phosphate metabolite of tenofovir, tenofovir diphosphate (TFV-DP) intracellular concentrations were simulated as previously described [22]. The first order elimination rate of emtricitabine triphosphate (FTC-TP) from the cellular compartment was calculated considering the half-life of FTC-TP [23]. The maximum uptake velocity (V_{max}) and maximum velocity of anabolism (k_m) were fitted to obtain a zero-order dose input rate (k_{in}) such that the mean intracellular concentration of the active metabolite at steady state ($C_{\text{ss,avg}}$) was in accordance to available clinical data following this equation:

$$k_{\text{in}} = (V_{\text{max}} \times C) / (k_m + C) \quad (9)$$

where C is the concentration in the plasma.

Values of $0.9 \pm 0.18 \text{ ng} \times \text{h/mL}$ and $18 \pm 3.6 \text{ ng/mL}$ for V_{max} and k_m were suitable to obtain $C_{\text{ss,avg}}$ values comparable to previously published data for oral administration [23]. The optimisation of the dose and release rates for emtricitabine and tenofovir considered not only plasma concentrations but also the intracellular FTC-TP and TFV-DP over the reported *in vitro* IC_{50} values of $127 \text{ fmol}/10^6 \text{ cells}$ and $150 \text{ fmol}/10^6 \text{ cells}$ [24, 25].

3 Results

The validation was conducted by comparing the mean simulated C_{trough} , maximum plasma concentration (C_{max}) and area under the concentration-time curve (AUC) with the available clinical data for oral administration and also for an existing LA intramuscular formulation of rilpivirine (600 mg; 100 mg/mL) (shown in Table 2 and Figure 1). For emtricitabine and tenofovir, the $C_{\text{ss,avg}}$ of FTC-TP and TFV-DP were compared with clinical data [23].

Although the commonly agreeable limits of variability for validation is 2-fold from the mean value, we used a more stringent value of 0.5-fold to enhance the accuracy of the predictions [26].

For intramuscular formulations, multiple dose and release rate combinations were simulated to obtain mean predicted C_{trough} greater than the defined cut-off. For tenofovir and emtricitabine, the dose and release rates for intramuscular injection were optimised such that the plasma concentrations were over the IC_{90} value and also the $C_{ss,avg}$ were over the intracellular IC_{50} .

A summary of the predicted AUC, C_{max} and C_{trough} for eight ARVs following optimal intramuscular injection is shown in Table 3.

3.1 Rilpivirine (RPV)

The validation of the PBPK model for rilpivirine was done against available clinical data for the oral formulation. The simulated mean AUC, C_{max} and C_{trough} values deviated +38.7%, +5.1% and +6.5%, respectively from the clinical data [27]. The PBPK model was additionally validated against an existing LA intramuscular formulation of RPV (600 mg; 100 mg/mL) [6]. The release rate from the intramuscular depot was predicted after matching the model to the clinical pharmacokinetic data. The drug release followed dose-dependent first order kinetics and the release rate was predicted to be between 0.0009 and 0.0012 h^{-1} (Figure 1). The mean values for AUC were 84.0 $ng \times h/mL$ vs. 83.38 ± 33.34 $ng \times h/mL$, C_{max} 96.7 ng/mL vs. 86.73 ± 30.51 ng/mL and C_{trough} 15.7 vs. 11.81 ± 6.3 ng/mL for clinical versus simulated data. After the validation step with both oral and LA intramuscular formulations, the optimal dose and release rates of both weekly and monthly injection regimens of rilpivirine were identified such that the plasma concentration is over the $PBIC_{95}$ value of 20.3 ng/mL [28]. A weekly dose of 60 mg with a release rate of 0.015 h^{-1} and a monthly dose of 250 mg with a release rate of 0.002 h^{-1} were predicted to maintain the plasma concentrations above the target cut-off.

3.2 Atazanavir (ATV)

The validation at steady-state for the oral administration of unboosted atazanavir resulted in mean predicted AUC, C_{max} and C_{trough} values which were -16.9%, -49.3% and +50.0% of those observed clinically [29]. An intramuscular dose of 1500 mg atazanavir was predicted to result in plasma concentrations after 30 days below the $PBIC_{95}$ (60 ng/mL) and was therefore deemed unsuitable for monthly injection. An intramuscular weekly dose of 400 mg however was predicted to achieve mean concentrations in excess of the $PBIC_{95}$ with a release rate of 0.009 h^{-1} [30].

3.3 Dolutegravir (DTG)

The predicted variation from the clinical values for mean AUC, C_{max} and C_{trough} in the validation of oral administration of dolutegravir were +18.7%, -5.3% and +68.2%, respectively [31]. Considering the $PBIC_{95}$ value of 64 ng/mL as the target concentration, the simulated intramuscular administration requires an estimated weekly dose of 20 mg with a release rate of 0.006 h^{-1} and a monthly dose of 105 mg with a release rate of 0.002 h^{-1} [32].

3.4 Emtricitabine (FTC)

The predicted variation from the clinical values in mean AUC, $C_{\max}$ and C_{trough} for oral emtricitabine were -3.5% , -34.0% and -28.1% , respectively [33] and the simulated mean $C_{\text{ss,avg}}$ of intracellular FTC-TP has a deviation of $+2.65\%$ from the clinical value (Table 4). The IC_{90} value of 50.2 ng/mL and intracellular IC_{50} of $127 \text{ fmol}/10^6 \text{ cells}$ were considered as target concentrations [25, 23]. The simulated intramuscular injection required a dose of 375 mg with a release rate of 0.009 h^{-1} for a weekly administration and 1500 mg with a release rate of 0.0015 h^{-1} for a monthly administration [23].

3.5 Efavirenz (EFV)

The validated PBPK model yielded AUC, $C_{\max}$ and C_{trough} values, which were $+13.7\%$, -20.2% and -3.4% compared to the clinical values [34]. In order to obtain plasma concentrations above the PBIC_{95} value of 126 ng/mL , a weekly intramuscular dose of 200 mg (release rate - 0.015 h^{-1}) and monthly dose of 800 mg (release rate - 0.007 h^{-1}) were required [30].

3.6 Etravirine (ETV)

The validated PBPK model with oral administration of etravirine resulted in mean AUC, $C_{\max}$ and C_{trough} values which were $+6.1\%$, -16.9% and $+21.0\%$ compared to the clinical values [35]. The intramuscular predictions indicate that etravirine may be suitable for weekly administration with a dose of 700 mg and release rate of 0.015 h^{-1} but not for monthly administration as the drug concentrations fall below the PBIC_{95} value even for an intramuscular dose of 1500 mg [30].

3.7 Raltegravir (RAL)

The predicted mean AUC, $C_{\max}$ and C_{trough} of orally administered raltegravir were $+35.4\%$, -20.5% and -23.1% compared to the clinical values, respectively [36]. To maintain the plasma concentrations above the IC_{95} value (15 ng/mL), the prediction suggested a dose of 200 mg (release rate - 0.005 h^{-1}) and 800 mg (release rate - 0.002 h^{-1}) may enable weekly and monthly intramuscular administration of raltegravir, respectively [37].

3.8 Tenofovir disoproxil fumarate (TDF)

The validated PBPK model for TDF predicted mean AUC, $C_{\max}$ and C_{trough} which were $+41.6\%$, $+78.0\%$, -67.9% compared to the clinical data, respectively [38]. The deviation of the intracellular $C_{\text{ss,avg}}$ was $+3.85\%$ compared to the clinical value (Table 4). 200 mg TDF with a release rate of 0.008 h^{-1} was predicted to provide a sufficient exposure following weekly intramuscular injection and 1300 mg with a release rate of 0.002 h^{-1} was an adequate dose for a monthly intramuscular administration, maintaining mean plasma concentrations above the IC_{90} and intracellular TFV-DP above the IC_{50} [24].

4 Discussion

Imperfect adherence to daily oral ARV formulations continues to hinder the efficacy of HIV therapy and PrEP. Alternative administration strategies are beginning to emerge that provide

opportunities for once monthly or even less frequent administration. A greater understanding of formulation characteristics is required to predict dose and release rates that provide adequate pharmacokinetic exposure relative to potency. This information will facilitate development of newer LA ARV formulations.

In this study, PBPK modelling was used to predict the pharmacokinetics of ARVs following intramuscular injection. The PBPK models were based on mathematical equations describing the human anatomy and physiology and the molecular processes regulating drug release and distribution. The PBPK models were initially validated for eight orally administered ARVs against available clinical data for oral formulations. Subsequently, intramuscular administration was simulated by adding an additional compartment to the model to represent the intramuscular depot. For this, a first order kinetics constant was applied to represent the diffusion of ARVs from the depot to the surrounding blood capillaries (as shown in Figure 2). This approach was validated comparing simulated pharmacokinetics of RPV following intramuscular injection versus available clinical data for the LA formulation. The primary objective of this study was to identify whether drug-specific doses and release rates from the depot predicted compatibility for intramuscular administration. To obtain the suppression of the viral replication, ARV C_{trough} should reach and be maintained at concentrations above the susceptibility of the virus to that drug. Where possible, dose and release rate were optimised such that the ARV C_{trough} remained above previously identified target values. Target concentrations were identified as equal to $PBIC_{95}$ which represent the ARV concentration that can inhibit 95% of the virus replication *in vitro*. Since emtricitabine and tenofovir disoproxil fumarate are characterised by very low protein binding (<4% and <0.7% respectively) IC_{90} values were considered (IC_{95} data not available). The intracellular concentrations of FTC-TP and TFV-DP were also used as target concentrations in the optimisation of intramuscular dose and release rates for emtricitabine and tenofovir. Although the $PBIC_{95}$ represents a rational pharmacodynamic cut-off and has been discussed extensively elsewhere, some ARVs do not exert a good relationship between trough concentrations and virologic efficacy [30]. In certain cases the clinical data indicate that the minimum effective concentrations for therapy might be considerably higher than the *in vitro* IC_{95} .

The validation of PBPK models is essential to identify the correct parameters for an accurate simulation of pharmacokinetics for each ARV. Since dissolved molecule rather than nanoparticle is assumed to predominate systemically after intramuscular administration of SDNs, the volume of distribution, metabolism and elimination were fixed irrespective of the route of administration. As such, it should be noted that the presented data are only applicable when this is assumed to be the case, and these models cannot be applied to technologies that change these parameters (e.g. nanocarrier systems such as nanoemulsions or polymeric nanoparticles). Each model prediction was carried out at steady-state to better represent the long term ARV pharmacokinetics and relevant clinical scenarios. The model was considered validated if the mean AUC of the simulation for each ARV was within $\pm 50\%$ compared to clinically reported values. The simulated values from all the included PBPK models were in agreement with available clinical data and were therefore used for prediction of pharmacokinetics after intramuscular administration. The simulations were

carried out for weekly and monthly administration and the maximum injectable dose was set to 1500 mg since doses above this have not previously been practical for intramuscular formulations [21]. Table 3 illustrates that the release rate was inversely proportional to the dosage, and a slower release rate was required for monthly administration.

Our PBPK models predicted that two NRTIs, emtricitabine and tenofovir, may be suitable for weekly or monthly administration. Importantly, emtricitabine and tenofovir are widely used in HIV treatment and prevention, and LA formulations of these agents would make possible multi-drug regimens that match current clinical paradigms for therapy. These agents also represent good candidates for monthly PrEP due to their excellent safety and efficacy profiles. Tenofovir alone, or tenofovir combined with emtricitabine, have already been shown to provide good protection against HIV infection in individuals at high risk of infection [3].

Considering NNRTIs, efavirenz and rilpivirine were both predicted to be suitable for monthly administration. Both have long half-lives, which are favourable for development of LA agents. The high systemic clearance of efavirenz limits the possibility of a monthly administration format when a dose limit of 1500 mg is applied. Conversely, both integrase inhibitors, dolutegravir and raltegravir, were predicted to be suitable for monthly administration. A low dose of 105 mg was predicted to be sufficient for monthly administration of dolutegravir if a formulation with optimised release rate can be developed. It should be noted that cabotegravir is chemically closely related to dolutegravir, and development of a dolutegravir LA formulation may therefore not be warranted. Our simulation indicated that raltegravir could potentially be developed as a monthly intramuscular injection with a dose of 800 mg. The optimised dose and release rate for unboosted atazanavir predicted feasibility of an injectable weekly option at 600 mg, but doses higher than 1500 mg would be required for monthly administration.

The optimisation of LA strategies should be based on a rational analysis of several pharmacokinetic factors. For many agents, it is likely that the pharmacokinetic variability after intramuscular administration will be reduced compared to oral formulations, due to the lower complexity of the absorption process and avoidance of intestinal transporters and first-pass metabolism. Therapeutic plasma concentrations were predicted to be achieved in approximately eight hours for emtricitabine and tenofovir, and ~24 h for the remaining ARVs after a single intramuscular injection (as shown in Figure 3). Since a day is needed to achieve therapeutic plasma concentrations for the other ARVs, a loading oral dose equivalent to the daily regimen may be necessary. This could represent a minor risk for the development of drug resistance and therapeutic failure, but PBPK modelling may help to inform the ongoing debate regarding some aspects of the need for an oral lead-in with the same drugs. The co-administration of multiple injectable ARVs is possible, but a rational selection of concomitant agents is needed to avoid drug-drug interactions. Studies to validate the utility of co-administration of the two existing LA drugs, rilpivirine and cabotegravir, are underway [39].

The PBPK approach is characterised by some intrinsic limitations due to existing knowledge gaps and the complexity of biological processes underpinning drug distribution. For

example, muscle tissues are well connected to the lymphatic circulation, and drugs with high lipophilicity tend to diffuse through lymphatics rather than blood vessels [9]. Consequently, higher accumulation of ARVs in lymph nodes might be possible, but there is a paucity of clinical data in this area for available LA formulations. Similarly, efflux and influx transporters are of clear importance to intestinal absorption, distribution and clearance of drugs, but there is currently very little data on whether these transporters (or metabolic enzymes) are expressed at the depot sites. Gender has been correlated with changes in plasma exposure in a recent clinical study of rilpivirine LA [40]. Although several suggestions have been put forward to explain this, the physiological and anatomical mechanisms characterising the gender differences have not been fully elucidated and consequently have not been included in our simulations.

Although the doses and release rates identified through these simulations set targets for optimal nanoparticle design, it is important to underscore that the technological challenges in achieving these formulation characteristics are not addressed in this work. Long term stability of the ARVs in the interstitial fluid present in the depot site is another important consideration. Since SDN strategies do not involve the use of a carrier with stable size and dissolution rates over time, the nanoparticle properties are likely to change over the dosing interval. Since this will alter the surface to mass ratio, an effect on ARV release kinetics with downstream effect on the overall pharmacokinetics cannot be ruled out. It may also be important to consider other SDN components, such as polymers and surfactants, which will also be gradually released over time [41]. The use of LA injections of ARVs might determine specific complications at the site of injection as described for other treatments; additionally given the prolonged systemic exposition of LA ARV and the inability to interrupt the drug in the event of side effects, a period of oral induction might be considered before initiating LA injections. For patients discontinuing the LA ARV therapy, an oral administration strategy should be considered in order to prevent resistance development due to the diminishing plasma concentrations [4].

5 Conclusion

PBPK models for the simulation of pharmacokinetics after LA administration were successfully developed and validated against clinical data for eight approved ARV drugs. The validated models were applied to identify theoretical optimal dose and release rates required to achieve therapeutic concentrations following intramuscular injection. This approach defines a new paradigm for the rational design of LA formulations incorporating pharmacological properties of candidate ARVs. ARVs with potential for reformulation into intramuscular depots were identified, assuming the technological complexities associated with reformulation can be overcome. Based on their pharmacokinetic properties, dolutegravir, efavirenz, emtricitabine, raltegravir, tenofovir and rilpivirine are potential candidates for once monthly injection. PBPK modelling represents an innovative pharmacological tool to inform drug and formulation design.

Acknowledgements

Source of Funding:

This work was supported by the National Institutes of Health (AI 114405-01).

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Key Points

- This study investigated the feasibility of applying mathematical models to candidate LA formulations for antiretrovirals in order to predict drug concentrations over time.
- Six antiretrovirals have been identified as potential therapeutic options for once monthly intramuscular administration, providing theoretical optimal dose and release rates.
- This modelling approach constitutes a new paradigm for the rational design of LA formulations, considering a thorough evaluation of the drug pharmacological properties and defining an innovative platform for the optimisation of future nanoformulations.

Fig 1.
Validation of the physiologically based pharmacokinetic strategy against clinical data for an existing rilpivirine sustained-release formulation (600 mg; 100 mg/mL) [6]

Fig 2.

The whole body physiologically based pharmacokinetic model with an additional compartment for intramuscular depot and the blood capillaries surrounding the depot. Each of the arrows represents the flow kinetics assumed between tissues in the model. The small intestine is divided into seven parts for effective absorption kinetics. Metabolism occurs in the liver and intestine. Unabsorbed drug is excreted through faeces and excretion occurs through the kidneys. The green line represents the kinetics of the release from the nanoparticulate intramuscular depot. IM – intramuscular, RV and LV - right and left ventricle respectively.

Fig 3.
Pharmacokinetics of monthly administration of antiretrovirals over a period of 6 months. a) Dolutegravir, b) Efavirenz, c) Emtricitabine, d) Raltegravir, e) Rilpivirine and f) Tenofovir

Table 1

Physicochemical properties, *in vitro* and population pharmacokinetic data of antiretrovirals

Drug Properties	Atazanavir	Dolutegravir	Efavirenz	Emtricitabine	Etravirine	Raltegravir	Rilpivirine	Tenofovir
Molecular weight	704	419	316	247	435	444	366	287
log P_{ow}	3.17	2.20	4.60	-0.43	5.20	0.58	4.32	1.25
Protein binding	86.0%	99.3%	98.0%	4.0%	99.0%	83.0%	99.7%	0.7%
pK_a	7.4	8.3	10.2	2.65	3.75	6.67	3.26	3.75
R	0.75 [13]	0.535 [42]	0.74 [43]	1.0 [33]	0.7 [44]	0.6 [45]	0.67 [27]	<i>b</i> ₁
PSA (Å²)	-	-	38.33	-	120.64	-	-	-
HBD	-	-	1	-	3	-	-	-
P_{app} (10⁻⁶ cm/s)	-	-	2.5 [43]	-	-	6.6 [45]	12 [27]	-
k_a (h⁻¹)	1.5 [13]	1.63 ± 1.03 [46]	-	1.0 [47]	-	-	-	1.03 [48]
F	68%	-	-	93%	-	-	-	20%
V_d (L/kg)	1.2 [13]	-	3.6	1.4 ± 0.3	^a 6.03	0.6	-	0.813 [38]
Metabolism								
CL/F (L/h)	^a 12.6 (10.5-15.4) [49]	1 (15%) [31]	-	-	-	4.62 [50]	-	-
Renal Clearance (L/h)	-	-	-	12.80 ± 5.34 [33]	-	3.6 [45]	-	11.3 ± 6.2 [51]
CYP2A6 CL_{int}	-	-	0.08 [43]	-	-	-	-	-
CYP2B6 CL_{int}	-	-	0.55 [43]	-	-	-	-	-
CYP1A2 CL_{int}	-	-	0.07 [43]	-	-	-	-	-
CYP3A4 CL_{int}	-	[†] 3.0 [52]	0.007 [43]	-	0.012 [53]	-	2.04 [27]	-
M2 CYP3A4 CL_{int}	-	-	-	-	0.00091 [53]	-	-	-
CYP3A5 CL_{int}	-	-	0.03 [43]	-	-	-	-	-
CYP2C19 CL_{int}	-	-	-	-	0.75 [53]	-	-	-
UGT1A1 CL_{int}	-	[†] 3.2 [52]	-	-	-	[‡] 12.4 [45]	-	-

CYPs induction	Atazanavir	Dolutegravir	Efavirenz	Emtricitabine	Etravirine	Raltegravir	Rilpivirine	Tenofovir
CYP3A4 Ind _{max}	-	-	6.5 [43]	-	2.5 [53]	-	-	-
CYP2B6 Ind _{max}	-	-	5.7 [43]	-	-	-	-	-
CYP3A4 Ind ₅₀	-	-	3.9 [43]	-	-	-	-	-
CYP2B6 Ind ₅₀	-	-	0.8 [43]	-	-	-	-	-

^a An average adult body weight of 70 kg was assumed

^b Value was an assumption (as the drug is similar to emtricitabine and tenofovir value was not available in the literature)

^c The units are in $\mu\text{min}/\text{mg}$

^d The units are in $\mu\text{min}/10^6$ hepatocytes; $\log P_{O/w}$ – Partition coefficient between octanol and water; pK_a – logarithmic value of the dissociation constant; R – blood-to-plasma drug ratio; PSA – polar surface area; HBD – number of hydrogen bond donors; P_{app} – drug permeability from apical to basolateral in Caco-2 cell monolayer;; K_d – absorption coefficient; F – absolute bioavailability; V_d – volume of distribution; CL/F – Apparent oral clearance; CL_{int} – intrinsic clearance ($\mu\text{min}/\text{pmol}$); CYP – cytochrome P450; UGT – uridine diphosphate glucuronosyltransferase; M2 – Second metabolite; Ind_{max} – induction maximum; Ind₅₀ – 50% induction; ‘-’ – data not available

Table 2
Validation of antiretrovirals at steady state: Clinical vs. Predicted AUC, Cmax and Ctrough

Drug	Dose (mg)	AUC (ng×h/mL)		C _{max} (ng/mL)		C _{trough} (ng/mL)	
		Clinical	^a Predicted	Clinical	^a Predicted	Clinical	^a Predicted
Efavirenz	600 OD	^a ₅₇ 1,150 ± 27,300 [34]	65,006 ± 16,383	4,000 ± 1,710 [34]	3,193 ± 699.7	2,380 ± 1,140 [34]	2,298 ± 671.0
Etravirine	200 BD	^a ₃ 3,713 ± 2,069 [35]	3,939 ± 1,249	451.3 ± 232.3 [35]	375.0 ± 106.1	235.9 ± 163.1 [35]	285.4 ± 100.5
Rilpivirine	25 OD	^a ₂ 589 ± 868.8 [27]	3,590 ± 1,256	203.8 ± 75.81 [27]	214.1 ± 53.9	89.85 ± 38.07 [27]	95.7 ± 49.5
Raltegravir	400 BD	^a ₇ 076 ± 4,071 [36]	9,584 ± 2,160	2,519 ± 1,930 [36]	2,002 ± 378.9	71 ± 50 [36]	54.6 ± 52.1
Atazanavir	400 OD	^a ₂₉ 303 ± 8,263 [29]	24,354 ± 4,307	5,358 ± 1,371 [29]	2,718 ± 392.2	218 ± 191 [29]	326.9 ± 150.0
Tenofovir	300 OD	^b ₃ 324 (41.2%) [38]	4,707 ± 1,004	326 (36.6%) [38]	580.2 ± 80.6	64.4 (39.4%) [38]	20.7 ± 17.7
Emtricitabine	200 OD	^b ₁₀ 100 (18%) [23]	9,747 ± 2,326	1,720 (16%) [23]	1,136 ± 236.3	73 (28%) [23]	52.5 ± 32.2
Dolutegravir	50 OD	^b ₅₃ 600 (27%) [31]	63,615 ± 8,802	3,670 (20%) [31]	3,474 ± 519.6	1,110 (46%) [31]	1,867 ± 309.0

^a Arithmetic Mean ± S.D.

^b Geometric Mean (% CV – coefficient of variation expressed as a percentage), AUC – area under the concentration-time curve, Cmax – maximum plasma concentration, Ctrough – trough plasma concentration, OD – once daily, BD – twice daily.

Table 3
Prediction of the dose and release rate of single intramuscular injection of antiretrovirals

Drug	Intramuscular Dose (mg)	Release rate (h ⁻¹)	Weekly/Monthly	AUC (μg×h/mL) [†]	C _{max} (ng/mL) [†]	C _{trough} (ng/mL) [†]	Cut-off limit (ng/mL)
Nucleoside Reverse Transcriptase Inhibitors (NRTIs)							
Emtricitabine	1,500	0.0015	Monthly	43.1 ± 15.4	94.2 ± 32.5	51.2 ± 41.3	50.2 [23] (IC ₉₀)
	375	0.009	Weekly	20.5 ± 8.9	199.4 ± 76.1	55.6 ± 26.4	
Tenofovir	1,300	0.002	Monthly	52.2 ± 15.4	99.2 ± 28.6	43.8 ± 17.2	18 [2] (IC ₉₀)
	200	0.008	Weekly	16.6 ± 7.1	155.6 ± 58.5	49.1 ± 23.0	
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)							
Efavirenz	800	0.007	Monthly	333.9 ± 99.3	850.4 ± 194.6	140.0 ± 50.8	126 [30] (PBIC ₉₅)
	200	0.015	Weekly	73.2 ± 22.1	546.5 ± 139.5	273.3 ± 81.8	
Etravirine	700	0.015	Weekly	43.4 ± 12.3	359.3 ± 80.7	126.1 ± 60.7	116 [30] (PBIC ₉₅)
Rilpivirine *	250	0.002	Monthly	32.9 ± 14.7	75.4 ± 30.1	22.6 ± 8.2	20.3 [28] (PBIC ₉₅)
	60	0.015	Weekly	10.8 ± 4.8	97.4 ± 33.3	26.1 ± 18.7	
Integrase Inhibitors (IIs)							
Dolutegravir	105	0.002	Monthly	95.6 ± 8.5	212.1 ± 18.1	65.7 ± 7.1	64 [32] (PBIC ₉₅)
	20	0.006	Weekly	24.2 ± 2.8	169.5 ± 18.1	101.7 ± 14.8	
Raltegravir	800	0.002	Monthly	26.1 ± 5.2	66.9 ± 13.1	15.7 ± 2.8	15 [37] (IC ₉₅)
	200	0.005	Weekly	8.23 ± 1.47	69.4 ± 12.1	30.1 ± 5.4	
Protease Inhibitors (PIs)							
Atazanavir	400	0.009	Weekly	38.9 ± 8.3	334.3 ± 70.7	114.8 ± 25.4	60 [30] (PBIC ₉₅)

* Note that this dose is not for the existing rilpivirine formulation

[†] Values are shown as arithmetic mean and standard deviation; AUC – area under the concentration-time curve, C_{max} – maximum plasma concentration, C_{trough} – trough plasma concentration, IC₉₀ – 90% inhibitory concentration, PBIC₉₅ – 95% protein binding adjusted inhibitory concentration

Table 4

Validation and prediction of intracellular concentration of emtricitabine triphosphate and tenofovir diphosphate

Active Drug	Intracellular $C_{ss,avg}$ (fmol/10 ⁶ cells)		Weekly/ Monthly	Intramuscular Prediction (fmol/10 ⁶ cells)		<i>In vitro</i> IC ₅₀ (fmol/10 ⁶ cells)
	Clinical	Simulated		Intracellular C _{trough}	Intracellular C _{max}	
Emtricitabine triphosphate	1124 ± 592 [23]	1,154 ± 456	Monthly	163.0 ± 124.6	228.2 ± 106.1	127 [25]
			Weekly	270.0 ± 130.0	431.8 ± 198.4	
Tenofovir diphosphate	150.7 ± 92.9 [22]	156.5 ± 59.5	Monthly	154.2 ± 46.5	164.6 ± 49.3	150 [24]
			Weekly	163.0 ± 59.0	174.9 ± 62.8	

C_{ss,avg} – mean steady state concentration, C_{trough} – trough concentration at the end of the duration, C_{max} – maximum concentration, IC₅₀ – 50% inhibitory concentration