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HIV Pre-Exposure Prophylaxis: Mucosal Tissue Drug Distribution of RT Inhibitor Tenofovir and Entry Inhibitor Maraviroc in a Humanized Mouse Model

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Abstract

Pre-exposure prophylaxis (PrEP) strategies utilizing anti-retroviral drugs show considerable promise for HIV prevention. However there is insufficient pharmacokinetic (PK) data on drug concentrations required for protection at the relevant mucosal tissues where the infection is initiated. Here we evaluated the utility of a humanized mouse model to derive PK data on two leading drugs, the RT inhibitor tenofovir (TFV) and CCR5 inhibitor maraviroc (MVC). Following oral dosing, both the drugs and the intracellular active TFV-diphosphate could be detected in vaginal, rectal and intestinal tissues. The drug exposures (AUC_{24hr}) were found to be higher in vaginal tissue compared to plasma with even higher levels detected in rectal and intestinal tissues. The overall trends of drug concentrations seen in humanized mice reflect those seen in the human thus establishing the utility of this model complementing the present non-human primate (NHP) models for future pre-clinical evaluations of promising HIV PrEP drug candidates.

Introduction

An estimated 34 million people worldwide were living with HIV in 2011 (2013). The same year 2.5 million new infections were reported, along with 1.7 million HIV related deaths (2013). While the current highly active antiretroviral therapy (HAART) regimens efficiently control the infection and delay progression to AIDS, there is no complete cure due to HIV viral latency (Ruelas and Greene, 2013). In addition, prolonged therapy can have other problems, including drug resistance and drug toxicity (Pennings, 2013). Preventive vaccines would be ideal to control the spread of HIV/AIDS, however no success thus far (Excler et al., 2014). Therefore, other approaches are urgently needed to confer protection. In this

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regard antiretroviral (ARV) drug-based pre-exposure prophylaxis (PrEP) strategies present a practical preventative alternative (Heneine and Kashuba, 2012; Nicol and Kashuba, 2010; Romano et al., 2013). Two types of PrEP strategies are currently being pursued with promising results (Heneine and Kashuba, 2012; Nicol and Kashuba, 2010; Romano et al., 2013). The first involves oral systemic administration of ARVs, while the second consists of topical vaginal or rectal application of ARV-containing gels with both strategies designed to prevent HIV sexual transmission by mucosal routes. Indeed, field clinical trials had shown promising results with the use of reverse transcriptase inhibitor Tenofovir (TFV) as a microbicide gel in preventing HIV vaginal transmission whereas TFV alone or in combination with Emtricitabine (Truvada) administered orally also provided significant protection (Abdool Karim et al., 2010; Grant et al., 2010).

While protection afforded by these above approaches is promising, inconsistencies were found regarding the levels of protection in different clinical trials (Abdool Karim et al., 2010; Baeten et al., 2012; Grant et al., 2010; Karim et al., 2011; Thigpen et al., 2012; Van Damme et al., 2012). In the CAPRISA trial, 44% protection was achieved with a TFV microbicide gel, whereas in the VOICE trial, which also used a TFV microbicide gel, the trial was discontinued before conclusion because of a lack of protection (Abdool Karim et al., 2010; McEnery, 2011; van der Straten et al., 2012). In the IPrEx trial, oral administration of Truvada showed a 44% reduction in HIV infection in men who have sex with men (MSM) (Grant et al., 2010). The FEM-PrEP trial, also with oral Truvada, was stopped early due to lack of efficacy in high-risk female population (Van Damme et al., 2012). The TDF2 study also evaluated oral Truvada in men and women and reported a 63% reduction in HIV infection acquisition (Thigpen et al., 2012). The Partners PrEP study, looking at serodiscordant couples, showed a 67% reduction in HIV-1 incidence with oral TFV and a 75% reduction with Truvada (Baeten et al., 2012). Additional clinical trials are being conducted in various test populations and results are being awaited (Cohen et al., 2013). The above conflicting results with regard to variable levels of protection are attributed to differences in ARV dosing regimens, trial designs and target populations, sexual practices and behavioral patterns affecting adherence. A major factor that contributes to protection against HIV is the proper drug concentration to be reached in the target mucosal tissues, such as vaginal and rectal tissue. Tissue drug concentrations are affected by variables such as ARV dose, mode and frequency of administration, tissue permeability and protein binding, as well as drug half-life. Consequently, pharmacokinetic (PK) studies that focus on the mucosal compartments are critical for the future success of PrEP strategies.

Animal models have played crucial role in therapeutic drug development to derive important preclinical data (Akkina, 2013; Veazey, 2013). With regard to assessing HIV PrEP strategies non-human primates (NHP) models have been extremely useful in setting the stage for early human clinical trials (Evans and Silvestri, 2013; Fennessey and Keele, 2013). For example, TFV was successfully evaluated as both oral and topical PrEP in rhesus macaque studies (Garcia-Lerma et al., 2010; Garcia-Lerma et al., 2008; Nuttall et al., 2012; Parikh et al., 2009). The HIV entry inhibitor maraviroc (MVC) was evaluated similarly in NHP (Forbes et al., 2011; Malcolm et al., 2013). However, a number of limitations are apparent with NHP models. First of all, they are expensive and use of large numbers of animals often is not feasible. In addition, SIV or SHIV, not HIV itself, must be applied as a challenge virus thus

precluding NHP use for testing drugs specifically developed against HIV. In this context, humanized mice (hu-mice) with a transplanted human immune system have become amenable to test various HIV prevention approaches (Akkina, 2013; Denton and Garcia, 2011). Two new generation humanized mouse models are currently available. The hu-HSC humanized mouse model such as RAG-hu employs engraftment of human hematopoietic stem cells (HSC) into newborn immunodeficient ($Rag1^{-/-}$ or $Rag2^{-/-}\gamma c^{-/-}$) mice whereas the BLT mouse model is derived by transplantation of human fetal thymus tissue, liver tissue and HSC (Akkina, 2013; Denton and Garcia, 2011). Both models harbor human immune cells in mucosal sites such as vaginal and rectal tissue, and are susceptible to HIV infection via these routes thus mimicking key aspects of viral mucosal transmission (Berges et al., 2008; Denton and Garcia, 2012; Nischang et al., 2012). Both topical and systemic PrEP strategies employing ARVs have been successfully tested using these models (Chateau et al., 2013; Denton et al., 2011; Neff et al., 2011; Neff et al., 2010). The drugs tested include TFV, MVC, RT inhibitor Emticitabine (FTC) and integrase inhibitor Raltegravir (RAL). Pharmacokinetic and pharmacodynamic (PK/PD) analyses of ARVs in relevant tissue compartments and blood plasma are important to ascertain the relationship between the systemic and local concentrations achieved after drug application and to correlate with antiviral efficacy (Romano et al., 2013; Thompson et al., 2013). Such data can inform the level and duration of effective drug dosing for HIV prevention. While a number of previous studies established the utility of humanized mouse models for efficacy testing of new PrEP strategies and more recently drug distribution in lymphoid tissues (Chateau et al., 2013; Choudhary et al., 2009; Denton et al., 2008; Denton et al., 2014; Denton et al., 2011; Neff et al., 2011; Neff et al., 2010), no PK studies using these models in the context of mucosal tissues have been described.

Here we evaluated the utility of hu-mice to derive PK data employing two different classes of ARVs - TFV and MVC. Our results show that both ARVs can be readily measured in blood plasma and mucosal compartments encompassing vaginal, rectal and intestinal tissues. The overall drug distribution kinetics in this system were found to be similar to humans thus validating this model for future studies employing more complex combinatorial PrEP dosing regimens.

Materials and Methods

Generation of humanized mice

Humanized BALB/c $Rag1^{-/-}$ or $Rag2^{-/-}\gamma c^{-/-}$ (Hu-HSC RAG-hu) mice were generated by engrafting human fetal liver-derived CD34+ hematopoietic progenitor cells (HPC) as previously described (Berges et al., 2008; Berges et al., 2006). Mice were maintained at the Colorado State University Painter Animal Center and all studies were approved by the CSU Institutional Animal Care and Use Committee (Protocol 11-3153A). Newborn mice were preconditioned by irradiating with a sub lethal dose of 350 rads and then injected intrahepatically with 0.5–1 $\times 10^6$ human CD34+ cells. Mice were screened for human cell engraftment at 10–12 weeks post-reconstitution. Peripheral blood was collected by tail bleed and red blood cells were lysed by the Whole Blood Erythrocyte Lysing Kit (R&D Systems, Minneapolis, MN).The white blood cell fraction was stained against the human pan-

leukocyte marker CD45 using hCD45-R-PE (Invitrogen) and FACS analyzed to determine the levels of human cell engraftment as previously reported (Berges et al., 2008; Berges et al., 2006). Mice with more than 50% human cell engraftment were used for experiments involving hu-mice to assure robust numbers of human cells being present.

Drug administration of antiretrovirals and sample collection

Female mice were administered with either Tenofovir or Maraviroc by oral gavage. Clinical formulations of these drugs in tablet form (Maraviroc (Selzentry) 150 mg, Pfizer Labs; Tenofovir disoproxil fumarate (Viread) 300 mg, Gilead Sciences), were freshly dissolved in sterile PBS prior to oral gavage. Mouse equivalent drug doses were calculated by using an interspecies allometric scaling factor of 12.3 to arrive at 61.5 mg/kg and 62 mg/kg doses for TFV and MVC respectively. Mice (three or five per group) received either TFV (1.23 mg per 20 gram mouse) or MVC (1.24 mg per 20 gram mouse) by oral gavage daily for 5 days. Plasma and tissue samples were collected for each drug following the last gavage at 2h, 8h, and 24h for TFV and at 4h, 12h and 24h for MVC. In addition, 48h plasma samples were collected for one group each of treated animals. All samples except 48h blood plasma were terminal and were collected during mouse necropsies. Tissue samples collected after drug administration consisted of vaginal, rectal and intestinal tissue samples. Tissue and plasma samples were snap frozen in liquid nitrogen within five minutes from the time of tissue collection. For negative controls, plasma and tissue samples were collected from untreated mice and were processed using the protocols described above. Samples were stored at −80°C until drug measurements.

Measurement of drug concentrations in plasma and tissue samples

Quantification of analytes was similar to previously published methods (Brown et al., 2011). Briefly, quantification of TFV concentrations in plasma was performed by protein precipitation and LC-MS/MS analysis with an isotopically-labeled internal standard (^{13}C TFV). TFV was eluted from a Waters Atlantis T3 (100 × 2.1mm, 3μm particle size) analytical column and an API-5000 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA) was used to detect the analytes. Data were collected using AB Sciex Analyst Chromatography Software (Analyst version 1.6.1). The dynamic range of this assay was 1-1000 ng/mL using a 1/concentration^2 weighted linear regression.

For measuring concentrations in mucosal tissues, TFV and TFV-DP was extracted from tissue homogenate by protein precipitation with isotopically-labeled internal standards (^{13}C TFV and ^{13}C TFV-DP). TFV was eluted from a Waters Atlantis T3 (100 × 2.1mm, 3μm particle size) analytical column, and TFV-DP was eluted from a Thermo Biobasic AX (50 × 2.1mm, 5μm particle size) analytical column. An API-5000 triple quadrupole mass spectrometer was used to detect all analytes. Data were collected using AB Sciex Analyst Chromatography Software (Analyst version 1.6.1). The dynamic range of this assay was 0.3-300 ng/mL of homogenate for each compound using a 1/concentration^2 weighted linear regression. Concentrations were ultimately converted into ng/mg (TFV) or fmol/mg (TFV-DP) tissue for final reporting.

To measure drug concentrations in plasma, MVC was extracted from samples using solid phase extraction with Varian BondElut C-18, 100 mg, 1CC cartridges. Plasma samples were quantified against the internal standard, alprazolam, on an Agilent 1200 series HPLC system using a Zorbax Eclipse XDB (50 × 4.6mm, 1.8μm particle size) analytical column. An Agilent 1100 MSD was used to detect the analyte and internal standard. The dynamic range of this assay was 1-1000 ng/mL.

For quantification from mucosal tissues, MVC was extracted from tissue homogenates using protein precipitation with the internal standard alprazolam. This resulting extract was analyzed on an Agilent 1200 series HPLC system using a Zorbax Eclipse XDB (50 × 4.6mm, 1.8μm particle size) analytical column. An Agilent 1100 MSD was used to detect the analyte and internal standard. The dynamic range of this assay was 0.007-7 ng/mg.

Statistical analysis

GraphPad Prism version 5 (GraphPad Software, USA) software was used for data analysis, figure generation, calculation of PK parameters and statistical analysis. PK data for TFV, TFV-DP and MVC in hu and non-hu mice were compared using Mann-Whitney two-tailed test. P values <0.05 were considered as significant. Measures of central tendency were expressed as median and inter-quartile range (IQR 25^{th}, 75^{th} percentile).

Results

TFV and TDP concentrations in plasma and mucosal tissue compartments

To compare the differential drug distribution and accumulation, TFV concentrations were measured in plasma and mucosal tissue compartments namely, vaginal, rectal and intestinal tissues at 2, 8, 24h post-last dose. In addition, plasma concentrations were also determined at 48h. The composite pharmacokinetic data on drug concentrations at various time points in different tissue compartments and plasma of hu-mice are presented in Fig 1 A1 whereas the data for individual hu-mice (5 mice per time point) for each of the compartments at different times are presented in Fig 1 B1-E1. Similarly, composite pharmacokinetic data and data for individual non-hu mice (3 mice per time point) are presented in Fig 1 A2 and Fig1 B2-E2, respectively. Data on TFV Cmax, Tmax, half-life ($t_{1/2}$) and area under the curve (AUC) are depicted in Table 1 for both hu and non-hu mice.

TFV was easily detected in all compartments at all time points (Fig 1 A-E). In hu-mice, highest concentrations in plasma were detected at 2h post dosing (Cmax 1,990 ng/ml) with a terminal elimination phase occurring by approximately 24h. The AUC_{48h} for plasma was 11,251 ng*h/ml. The median $t_{1/2}$ in plasma was 17h. In vaginal tissues, Cmax was at 2h (729 ng/g) with a gradual elimination by 24h. Vaginal tissue AUC_{24h} was 14,946 ng*h/g and TFV concentration in this tissue compartment was higher than in plasma at 8h and 24h. Half-life of TFV in vaginal tissue was 9h. Rectal tissue Cmax was 56,732 ng/g at 2h with an AUC_{24h} of 1×10^6 ng*h/g and $t_{1/2}$ of 13.5h. In intestinal tissue, Cmax was 74,677 ng/g at 2h. The AUC was 1.43×10^6 ng*h/g and tissue $t_{1/2}$ was 13.9h. The overall TFV exposure indicated by AUC_{24h} was the highest in intestinal tissue followed by rectal tissue, vaginal tissue and plasma. The AUC_{24h} tissue:plasma ratios for TFV were 1.5 for vaginal, 99 for

rectal and 141.7 for intestinal tissue. TFV exhibited higher exposures in rectal and intestinal tissue as a group, with concentrations one to two logs higher compared to vaginal tissue and plasma at all time points analyzed (Fig 1, Table 1). Half-life values of 13.5h and 13.9h in rectal and intestinal tissue respectively suggest similar elimination kinetics from these two tissues with slower elimination rate compared to vaginal tissue ($t_{1/2}$ 9h). In comparative experiments using non-hu mice, highest TFV concentrations in plasma were also detected at 2h post dosing (median Cmax 1,550 ng/ml) with a terminal elimination phase by 24h. The AUC_{48h} for plasma was 17,278 ng*h/ml. The median $t_{1/2}$ in plasma was 15h. In vaginal tissues, Cmax was at 8h (785 ng/g) with a gradual elimination by 24h. Vaginal tissue AUC_{24h} was 9,671 ng*h/g and TFV concentration in this tissue compartment was lower than in plasma at 2h and similar to plasma at 8h and 24h. Half-life of TFV in vaginal tissue was 6.3h. Rectal tissue Cmax was 76,520 ng/g at 8h with an AUC_{24h} of 1.3 $\times 10^6$ ng*h/g and $t_{1/2}$ of 53h. In intestinal tissue, Cmax was 50,334 ng/g at 8h. The AUC was 1×10^6 ng*h/g and $t_{1/2}$ was 61.6h. The overall TFV exposure in non-hu mice, indicated by AUC_{24h} was the highest in rectal tissue followed by intestinal tissue, plasma and vaginal tissue. The AUC_{24h} tissue:plasma ratios for TFV in non-hu mice were 0.7 for vaginal, 95 for rectal and 77.5 for intestinal tissue. As in hu-mice, non-hu mice TFV showed higher exposures in rectal and intestinal tissue as a group, with concentrations one to two logs higher compared to vaginal tissue and plasma (Fig 1, Table 1). Higher half-life values of 53h and 61.6h in rectal and intestinal tissue respectively were seen in non-hu-mice whereas the $t_{1/2}$ 6.3h seen in vaginal tissue was lower than that in hu-mice. The plasma TFV PK values between the hu and non-hu mice at some time points were found to differ significantly ($p<0.05$) unlike in issues.

The prodrug TFV is metabolized into its active form TFV-DP intracellularly in HIV target tissues and cell populations. Accordingly, the levels of TFV-DP were measured in different mucosal tissues (Fig 2 and Table 1). In hu-mice, the TFV-DP Cmax was at 2h in rectal tissues (604ng/g) and its AUC_{24h} was 70,194ng*h/g. The $t_{1/2}$ of TFV-DP in rectal compartment was 22.2h. In intestinal tissue TFV-DP Cmax was 8,841 ng/g at 8h whereas the AUC_{24h} was 172,167 ng*h/g. The intestinal TFV-DP $t_{1/2}$ was 3.5h. The TFV-DP concentrations could not be measured in vaginal tissues consistently as it was detected only once each at 2h and 24h. This is in line with difficulties noted in detection of low TFV-DP concentrations in previous human studies (Brown et al., 2011; Vourvahis et al., 2008). Overall, the intestinal TFV-DP exposure was found to be higher than in the rectal compartment indicated by the AUC_{24h} intestinal:rectal tissue ratio of 2.45. TFV-DP concentrations were also assessed in non-hu-mice for comparison. The TFV-DP Cmax was at 24h in rectal tissue (198 ng/g) and its AUC_{24h} was 3,188 ng*h/g. The $t_{1/2}$ of TFV-DP in rectal compartment could not be calculated due to no elimination seen at 24h. In intestinal tissue, Cmax was 119 ng/g at 8h, the AUC_{24h} was 2,414 ng*h/g and the $t_{1/2}$ was 45.3h. Overall, in non-hu mice the rectal TFV-DP exposure was found to be higher than in the intestinal compartment indicated by the AUC_{24h}. Similar to that in hu-mice above, TFV-DP could not be detected in vaginal compartment. TFV-DP PK differences in hu vs. non-hu mice were statistically significant ($p<0.05$) at all time points in both rectal and intestinal compartments with the exception of 2h in the intestinal tissue.

MVC concentrations in plasma and mucosal tissue compartments

The HIV entry inhibitor MVC acts extracellularly by interfering with viral binding to the CCR5 co-receptor. In the first set of experiments, non-hu mice were used (5 per each time point) whereas in the second set of experiments hu-mice (3 per time point) were used to determine if any differences exists between the PK values. The drug concentrations were measured in plasma and vaginal, rectal and intestinal mucosal tissue compartments at 4, 12, 24h post-last dose. In addition, plasma concentrations were also determined at 48h. The composite PK data on MVC drug concentrations at various time points in different tissue compartments and plasma are presented in Fig 3A1 (hu-mice) and 3A2 (non-hu-mice), whereas the data for individual mice for each of the compartments are presented in Fig 3 B1-E1 (hu-mice) and Fig 3 B2-E2 (non-hu-mice). PK parameters Cmax, Tmax, $t_{1/2}$ and AUC are shown in Table 2.

MVC was detected in all compartments at all time points analyzed. For non-hu mice, highest concentrations in plasma post-last dose were detected at 4h (Cmax 113 ng/ml). The AUC_{48h} for plasma was 3,280 ng*h/ml. The composite $t_{1/2}$ in plasma was 20.1 hrs. In vaginal tissue Cmax was at 4h (1,764 ng/g) with a gradual elimination up to 12h. Vaginal tissue AUC_{24h} was 11,239 ng*h/g and MVC was detected at higher concentrations than in plasma at 4 h, 12h and 24h. The $t_{1/2}$ of MVC in vaginal tissue was 3.7h. Rectal tissue MVC kinetics was characterized by Cmax of 3,785 ng/g at 4h, AUC_{24h} of 69,301 ng*h/g and $t_{1/2}$ of 2.9h. In intestinal tissue MVC Cmax was 18,194 ng/g at 4h, AUC_{24h} 134,883 ng*h/g and $t_{1/2}$ was 2.7h. The overall MVC exposure, indicated by AUC_{24h} was the highest in intestinal tissue followed by rectal tissue, vaginal tissue and plasma. The AUC_{24h} tissue:plasma ratios for MVC were 3.65 for vaginal, 22.5 for rectal and 43.8 for intestinal tissue.

With regard to PK of MVC in hu-mice versus non-hu mice, the overall tissue to plasma distribution trends were similar, with intestinal tissue showing the highest exposure (AUC_{24h} 140,159 ng*h/g), followed by rectal tissue (AUC_{24h} 32,690 ng*h/g), vaginal tissue (AUC_{24h} 2,461 ng*h/g) and plasma (AUC_{24h} 488.6 ng*h/ml, AUC_{48h} 755 ng*h/ml), Concentration of MVC at 4h (Cmax) in hu-mice was similar to that in non-hu mice in plasma. The MVC concentrations in rectal and intestinal tissues were higher in hu vs. non-hu mice. Significant difference was seen in plasma wherein the $t_{1/2}$ was 20 times lower in hu vs. non-hu mice (1h vs. 20h), while the $t_{1/2}$ in tissue compartments were slightly lower. Also, lower drug exposure (AUC_{24h}) was seen in hu-mice, with the exception of intestinal tissue. However, the observed differences in drug concentrations between hu-mice and non-hu mice were not found to be statistically significant (Mann-Whitney two-tailed test) except at 4h for intestinal tissue ($p < 0.05$) and 12h for vaginal tissue ($p < 0.05$).

Discussion

Nearly all present PrEP strategies employ currently approved ARTs for HIV prevention (Thompson et al., 2013). However, the doses being used for treating the patients do not accurately represent preventive doses necessary for full protection against HIV. Therefore, protective drug concentrations at the mucosal transmission target sites such as vaginal and rectal tissue must be defined to inform optimal dosing. Derivation of this much needed data by experimental studies will represent a significant advancement in the PrEP arena.

Here we utilized a humanized mouse model (RAG-hu) susceptible to HIV mucosal transmission to assess the drug concentrations following oral dosing with two leading PrEP candidates with different modes of action, RT inhibitor TFV and entry inhibitor MVC. Since concurrent measurements after single dosing may under or overestimate true tissue exposures due to different distribution characteristics in tissues compared with plasma, we measured drug concentrations after multiple dosing at steady state kinetics (Thompson et al., 2013). Our results showed that both TFV and MVC can be readily measured in blood plasma, vaginal, rectal and intestinal tissues. While there were number of previous studies in the human, this is the first experimental study that simultaneously measured the respective drug concentrations in multiple mucosal tissues to correlate their values with those in plasma (Romano et al., 2013; Thompson et al., 2013).

Previous studies on TFV in hu-mice showed protection against HIV vaginal challenge following sytemic and topical drug application (Chateau et al., 2013; Denton et al., 2008; Denton et al., 2011). Here with oral dosing, peak concentrations of TFV were detected at 2h after last dose in each of the compartments tested with a terminal elimination phase at 24-48h. The TFV half-life in blood plasma in hu-mice was comparable to that seen in an earlier human trial (Kearney et al., 2004). The overall drug exposure determined by AUC_{24hr} for TFV in vaginal tissue was found to be 1.5 higher than in blood plasma and two logs higher than in blood plasma in rectal and intestinal tissue. This is further illustrated by the tissue: plasma AUC_{24h} ratios (T:P ratio) (Fig 4), which were 1.5, 99.0 and 141.7 for vaginal, rectal and intestinal tissues respectively, indicating significantly higher drug exposure in vaginal and rectal tissues relative to plasma. This observed differential accumulation in various tissues is in agreement with the data from multiple human studies and could be due to multiple factors that include distinct mucosal expression and localization of drug transporters (Nicol et al., 2013; Romano et al., 2013; Thompson et al., 2013).

Post-uptake, the prodrug TFV is converted intracellularly to its pharmacologically active form TFV diphosphate (TFV-DP) inhibiting HIV-RT. As seen with it's prodrug, the TFV-DP exposure was also higher in intestinal tissue than in rectal tissue as depicted by higher AUC_{24h} (172,167 ng*h/g vs. 70,194 ng*h/g) and T:P ratio (141.7 vs. 99). In contrast, the half-life of TFV-DP was higher in rectal tissue (22.2h) compared to the intestinal tissue (3.5h). Differences in TFV-DP concentrations in rectal and intestinal compartments could be due to distinct expression and activity levels of native kinases needed for TFV to TFV-DP conversion, and/or differences in tissue expression of drug transporters, in addition to other factors influencing the PK of the prodrug TFV (Nicol et al., 2013; Romano et al., 2013; Thompson et al., 2013).

Consistent with human studies, higher concentrations of TFV were seen in hu-mouse mucosal tissues compared to blood plasma (Dumond et al., 2007; Kwara et al., 2008; Vourvahis et al., 2008). The overall TFV tissue exposures were also similar to that in the human as depicted by AUC_{24h} tissue:plasma ratios in vaginal, rectal and intestinal tissues (Patterson et al., 2011). Sustained TFV and TFV-DP concentrations were seen in the rectal compartment similar to a previous human study (Patterson et al., 2011). Long lasting intracellular TFV-DP levels seen in hu-mice are also akin to those seen in another previous

human trial (Hawkins et al., 2005). The extended half-life seen in the rectal tissue is advantageous for protection in MSM and this was documented in iPrEX study wherein it was found that individuals that did not fully comply with daily recommended dosing were still protected from HIV infection (Grant et al., 2010). These findings taken together suggest that overall, the relative PK trends of TFV and its active metabolite TFV-DP in hu-mice are similar to that seen in human studies and clinical trials. Moreover, the hu-mice data further confirm the favorable PK profile of TFV for its use as a PrEP agent.

With regard to the PK profiles of TFV and TFV-DP in hu- versus non-hu mice, while the overall AUC_{24h} trends were similar with drug concentrations being higher in intestinal, rectal tissues than in plasma, differences were also noted in tissue exposure levels, Tmax and $t_{1/2}$. For example, the TFV AUC_{24h} tissue:plasma ratios in vaginal and intestinal tissue of hu-mice were 2 fold higher compared to non-hu mice whereas the $t_{1/2}$ was longer in rectal and intestinal tissue of non-hu mice (53h and 61.6h, respectively) relative to that in hu-mice (13.5h and 13.9h). With TFV-DP, one to two log higher AUC_{24h} was seen in rectal and in intestinal tissues of hu-mice whereas it's $t_{1/2}$ was longer in non-hu mice in both intestinal (45.3h versus 3.5h) and rectal (no elimination phase was detected by 24h, compared to 22.2h $t_{1/2}$ in hu-mice) compartments compared to hu-mice (Table 1). While not entirely clear, the observed PK differences between hu- versus non-hu mice can be partly attributed to the absence of circulating lymphoid cell population, and thus drug clearance in the blood compartment as well as the mucosal tissues of non-humanized mice.

With respect to the entry inhibitor MVC, we have previously shown that its oral administration at allometric dosing levels is fully protective against HIV vaginal challenge in hu-mice (Neff et al., 2010). MVC exerts its antiviral action extracellularly in contrast to the RT inhibitor TFV which gets converted to its active form intracellularly. Therefore, in our initial experiments we analyzed MVC concentrations in non-hu mice. Maximum MVC concentrations were reached at 4h in plasma and in all the mucosal tissues. The highest exposure to MVC was seen in intestinal tissue (AUC_{24h} T:BP ratio of 43.8), followed by rectal and vaginal tissue (AUC_{24h} ratios of 22.5 and 3.6) The PK trends seen here were comparable to those in human studies(Brown et al., 2011; Dumond et al., 2009). As indicated by AUC_{24h} tissue:plasma ratios, there were higher exposures in vaginal and rectal tissue compartment to that in blood plasma, similar to that seen in previous human clinical trials (Brown et al., 2011; Dumond et al., 2009). In addition, blood plasma Tmax, elimination rate and AUC_{12h} were similar to those found in human studies (Abel et al., 2008; Dumond et al., 2009). Sustained high rectal drug concentrations up to 12h correlated well with those found in an earlier human study (Brown et al., 2011).

We next determined the MVC pharmacokinetics in hu-mice to see if any differences exist when compared with non-hu mice. Similar to the results seen in non-hu mice, maximum drug concentrations were reached at 4h in all compartments analyzed. Higher drug exposure (AUC_{24h}) was also detected in rectal and intestinal tissues compared to vaginal tissue. Hower a significant difference was the much shorter half-life seen in blood plasma of hu-mice versus non-hu mice (1h vs. 20h). This could be attributable to MVC binding to the CCR5 co-receptor on human cells in the blood and tissue compartments of hu-mice. AUC_{24h} in plasma, vaginal and rectal tissues in non-hu-mice were found to be higher than in hu-mice

most likely due to the absence of human cells that would have sequestrated most of the drug in the blood cellular compartment. Higher tissue:plasma AUC_{24h} ratios were seen in hu-mice compared to non-hu mice for all tissues (Fig 5). This is not unexpected due to lower plasma MVC half-life seen in hu-mice. Relative to that seen in human studies, the AUC_{24h} tissue:plasma ratios were found to be higher in hu-mice. For vaginal tissue, MVC AUC_{24h} VT:BP ratio was 5 in hu-mice compared to 1.9 seen in the human whereas in rectal tissue, the RT:BP AUC ratio was 66.9 relative to the 8-26 range seen in the human. Nevertheless, the overall MVC exposure trends of higher drug concentrations seen in mucosal tissues versus blood plasma are similar between hu-mice and the human. The vaginal concentration of MVC necessary for full HIV prevention in human clinical studies is not currently known. Therefore the concentrations measured in hu-mice wherein MVC was shown to be fully protective against HIV vaginal challenge should inform future dosing strategies in the human (Neff et al., 2011). The overall excellent oral bioavailabilty and high genital tract exposure of MVC relative to plasma as demonstrated in these studies lends further support for it being an ideal candidate for oral PrEP. However in comparison with TFV and its active metabolite TFV-DP, MVC half-life in tissue compartments is much shorter and thus will require consistent dosing and more patient compliance in terms of adherence.

For both the ARVs analyzed, drug concentrations varied widely between individual mice at some time points although the composite PK trends were similar. This is not unlike that observed in previous NHP and human studies wherein a wide degree of inter and intra-subject variability was seen (Brown et al., 2011; Dumond et al., 2009; Dumond et al., 2007; Evans and Silvestri, 2013; Fennessey and Keele, 2013; Patterson et al., 2011; Vourvahis et al., 2008). While we used hu-mice with robust human cell reconstitution, their levels in individual mice could have partly played a role. However, this appears unlikely since wide variations in drug concentrations were seen in non-hu-mice as well.

With regard to the PK studies like these in the PrEP context, the importance of using hu-mice versus non-hu-mice is apparent. This is illustrated by differences such as MVC plasma half-life being lower in hu-mice compared to non-hu mice. Additionally for compounds such as TFV which function intracellularly after phosphorylation, use of hu-mice harboring human cells is also more appropriate. Based on the overall data from above, hu-mice susceptible to HIV mucosal transmission can be exploited to assess ARV PK parameters. In addition to complementing results from NHP models, data derived in hu-mice on novel drugs would help inform more detailed subsequent macaque studies where appropriate. Drug concentrations determined to confer protection against HIV itself in hu-mice can be extrapolated to the human to inform HIV prevention doses of novel ARVs for prophylactic purposes.

Since the use of a single drug may not be adequate to confer full protection in a global setting, future oral HIV PrEP strategies will most likely involve a combination of ARVs with different mechanism of action for complete efficacy. However, whether the combinatorial ARVs would retain similar tissue distribution and overall kinetics compared to when applied singly needs to be determined. Such combinatorial studies can be conducted in hu-mice to define PK/PD parameters of various multi-drug regimen approaches to assess potential additive, synergistic and antagonistic effects between different classes of ARVs. In

addition, adherence issues of PrEP use and efficacy can also be simulated in hu-mice to derive useful preclinical data (Adams et al., 2013). It is also clear from the present data and previous reports that drug distribution and kinetics in different tissues vary (Romano et al., 2013; Thompson et al., 2013). This could be due to multiple factors which include target cell drug influx and efflux properties, differential mucosal expression and distribution of specific drug transporters and distinct expression of cytochrome P450 (CYP) and other drug metabolizing enzymes in mucosal compartments (Nicol et al., 2013; To et al., 2013). Such parameters can be experimentally evaluated in the humanized mouse model in the context of the human immune cell populations targeted by HIV. The preclinical knowledge gained from such in vivo studies addressing these questions will be very useful for more informed clinical trials of new PrEP strategies

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Research highlights

- First study in a hu-mouse model to determine pharmacokinetics of anti-HIV drugs in mucosal tissues
- In vivo analysis of anti-HIV drugs used for HIV pre-exposure prophylaxis
- Tenofovir and maraviroc showed higher levels of accumulation in mucosal tissues versus plasma
- Hu-mice are more appropriate for ARV drug PK analysis for HIV PrEP studies than non-hu mice
- Data from hu-mice PK studies will complement those from non-human primates

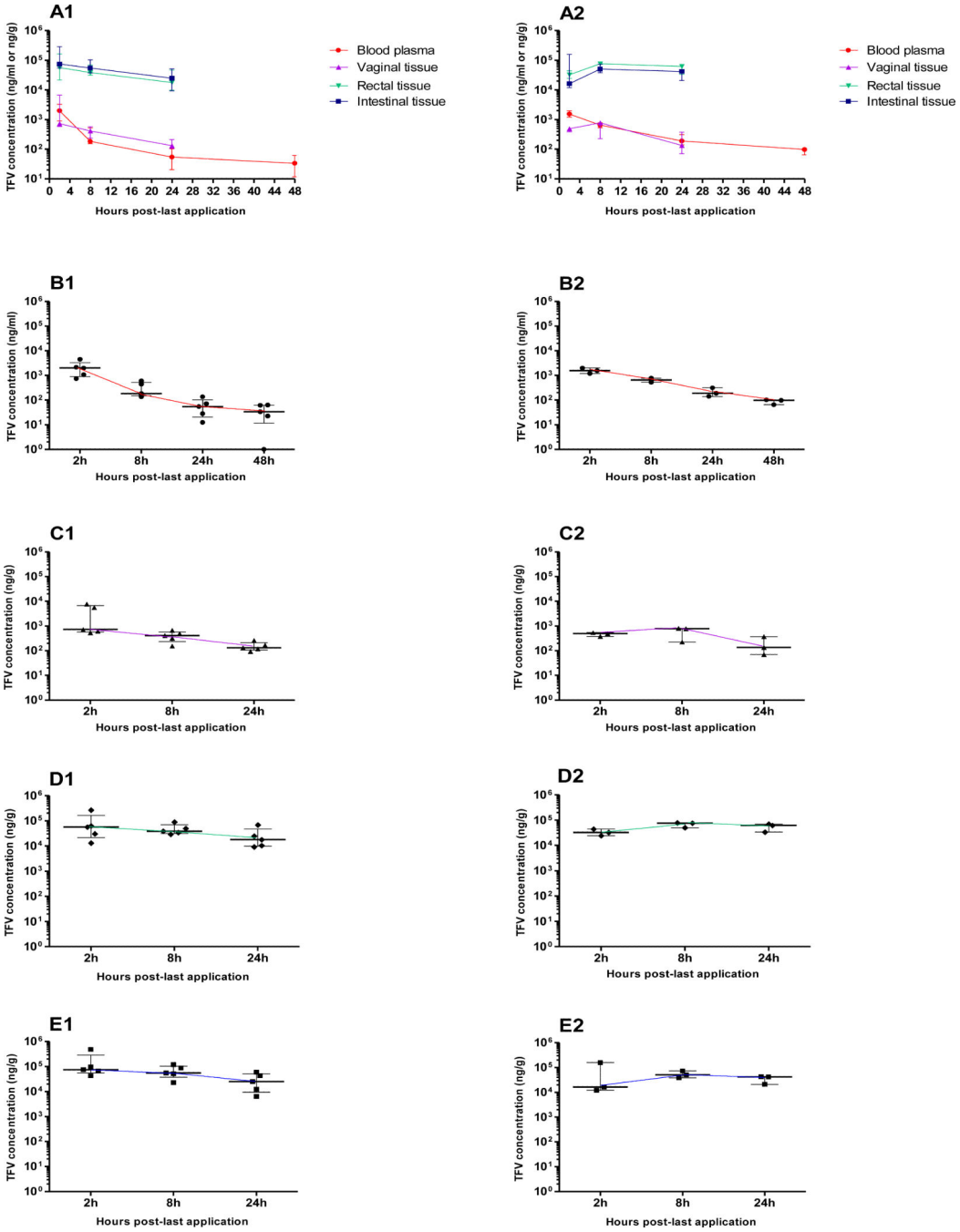

Figure 1. PK analysis of orally administered TFV in hu- and non-hu mice

Mice were administered TFV by oral gavage (human equivalent dose 61.5 mg/kg) for 5 days. Post-last dose, plasma and tissue samples were collected at different time points and drug concentrations were determined (ng/ml or ng/g). A1 (hu-mice), A2 (non-hu mice). Composite medians and interquartile ranges (IQR) for plasma, vaginal, rectal and intestinal tissue (colon). B1-E1 (hu-mice), B2-E2 (non-hu mice). Individual data points (n=5 hu-mice, n=3 non-hu mice) for plasma (B), vaginal (C), rectal (D) and intestinal tissue (colon) (E) with composite medians (IQR) shown.

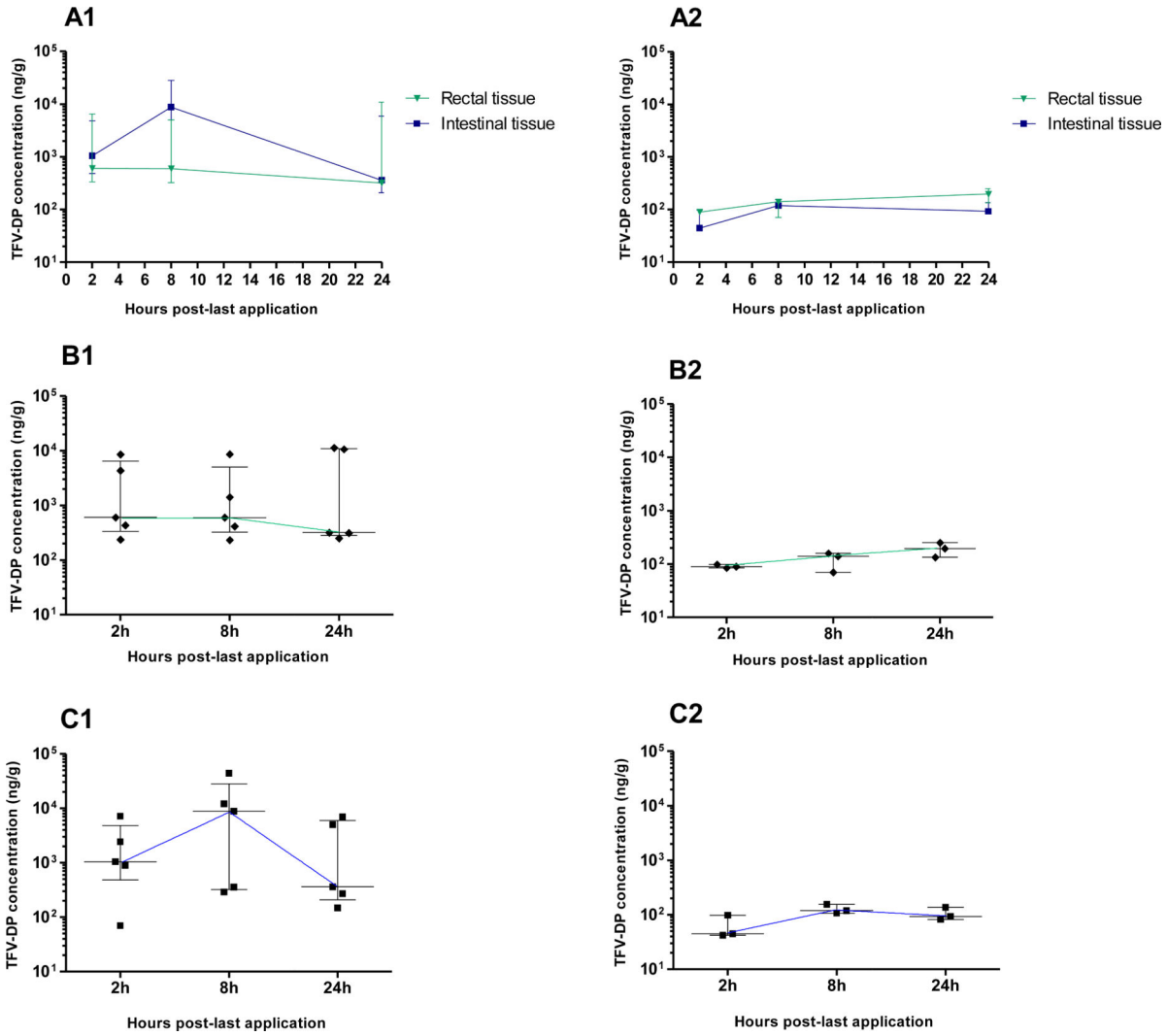

Figure 2. PK analysis of TFV DP in hu- and non-hu mice

TFV was gavaged as in Fig 1 above. Intracellular, diphosphorylated metabolite of TFV (TFV-DP) was detected and quantified in tissues at different times post-last dose. A1 (hu-mice), A2 (non-hu mice). Composite median, IQR for TFV DP in rectal and intestinal tissue (colon). B1,C1 (hu mice), B2,C2 (non-hu mice). Individual data points shown (n=5 hu mice, n=3 non-hu mice) with composite median, IQR in rectal (B) and intestinal tissue (C).

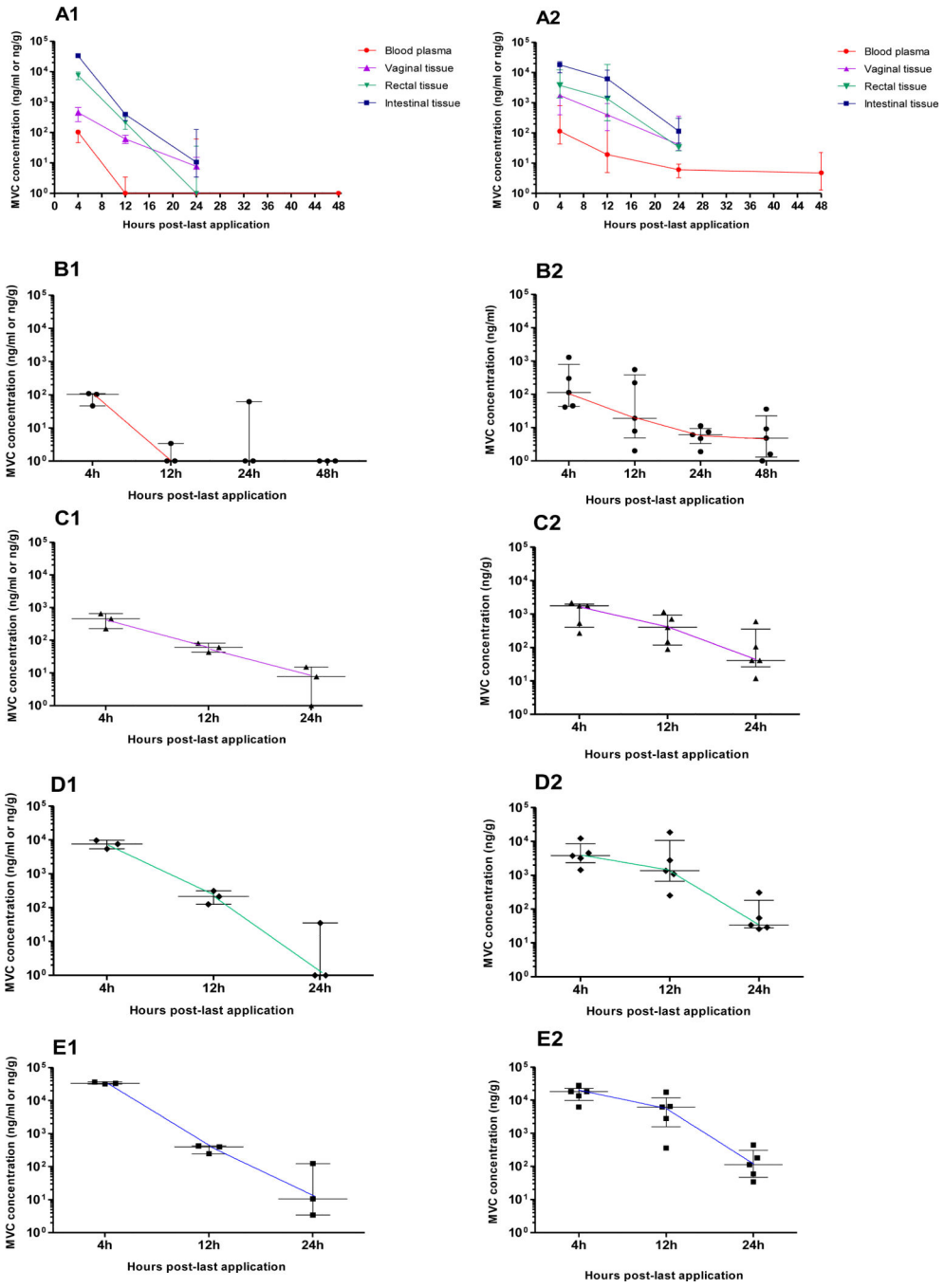

Figure 3. PK analysis of orally administered MVC in hu- and non-hu mice

Mice were administered MVC by oral gavage (human equivalent dose 62 mg/kg) for 5 days. Post-last dose, plasma and tissue samples were collected at different time points and drug concentrations were determined (ng/ml or ng/g). A1 (hu mice), A2 (non-hu mice). Composite medians and interquartile ranges (IQR) for plasma, vaginal, rectal and intestinal tissue (colon). B1-E1 (hu mice), B2-E2 (non-hu mice). Individual data points (n=3 hu-mice, n=5 non-hu mice) for plasma (B), vaginal (C), rectal (D) and intestinal tissue (colon) (E) with composite medians (IQR) shown.

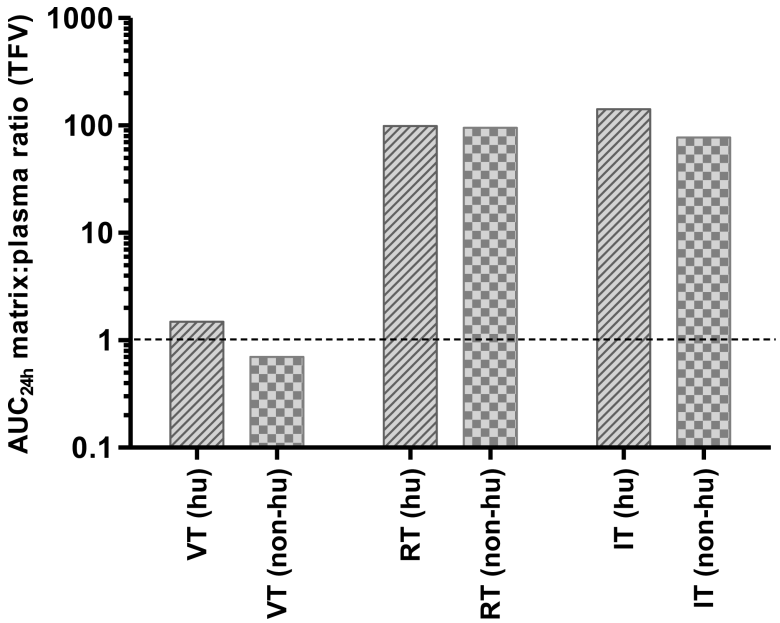

Figure 4. Tissue to blood plasma AUC_{24h} ratios of TFV

Tissue to plasma ratios were calculated for AUC_{24h}. On the y axis, 1 indicates a line of unity, where mucosal tissue exposure is similar to blood plasma. Values above the line of unity indicate higher drug exposure at mucosal sites compared to plasma. VT-vaginal tissue, RT-rectal tissue, IT-intestinal tissue (colon).

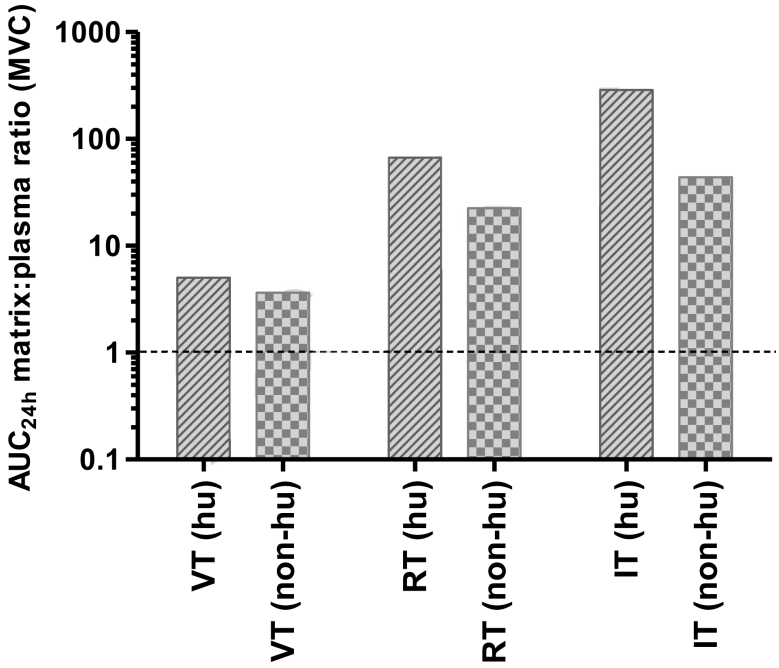

Figure 5. Tissue to blood plasma AUC_{24h} ratios of MVC

Tissue to plasma ratios were calculated for AUC_{24h}. On the y axis, 1 indicates a line of unity, where mucosal tissue exposure is similar to blood plasma. Values above the line of unity indicate higher drug exposure at mucosal sites compared to plasma. VT-vaginal tissue, RT-rectal tissue, IT-intestinal tissue (colon).

Table 1

PK parameters for Tenofovir and Tenofovir Diphosphate in humanized and non-humanized mice.

TFV (hu mice)	Plasma	Vaginal tissue	Rectal tissue	Intestinal tissue
Cmax *	1,990	729	56,732	74,677
Median (IQR)	(898 – 3,310)	(581 – 6,739)	(21,746 – 164,561)	(55,008 – 288,570)
Tmax	2h	2h	2h	2h
AUC †	11,251	14,946	1×10^6	1.43×10^6
$t_{½}$ ‡	17	9	13.5	13.9

TFV-DP (hu mice)				
Cmax *	NA	BLQ¥	604	8,841
Median (IQR)			(336 – 6,466)	(322 – 28,175)
Tmax	NA	/	2h	8h
AUC †	NA	/	70,194	172,167
$t_{½}$ ‡	NA	/	22.2	3.5

TFV (non-hu mice)				
Cmax *	1,550	785	76,520	50,334
Median (IQR)	(1,200-1,990)	(224 - 819)	(50,493 - 78,415)	(38,112 - 72,930)
Tmax	2h	8h	8h	8h
AUC †	17,278	9,671	1.3×10^6	1×10^6
$t_{½}$ ‡	15	6.3	53	61.6

TFV-DP (non-hu mice)				
Cmax *	NA	BLQ	198	119
Median (IQR)				
Tmax	NA	/	24h	8h
AUC †	NA	/	3,188	2,414
$t_{½}$ ‡	NA	/	/□	45.3

BLQ – below the limit of quantification; NA - not applicable

*Median (interquartile range, IQR), ng/ml or ng/g

†Area under the curve $(AUC)_{48h}$ for plasma (ng * h/ml); AUC_{24h} for tissues (ng * h/g)

‡Half-life ($t_{½}$, h)

¥TFV-DP detected in 2 samples in humanized mice – once at 2h and once at 24h

□no elimination phase

Table 2

PK parameters for Maraviroc in humanized and non-humanized mice.

MVC (hu-mice)	Plasma	Vaginal tissue	Rectal tissue	Intestinal tissue
Cmax *	102	459	7,577	33,462
Median (IQR)	(46 – 109)	(228 – 659)	(5,492.6 – 9,769)	(31,958 – 36,819)
Tmax	4h	4h	4h	4h
AUC †	755	2,461	32,690	140,159
$t_{½}$ ‡	1	2.6	1.5	1.2
MVC (non-hu mice)				
Cmax *	113	1,764	3,785	18,194
Median (IQR)	(43 - 797)	(403 – 1,982)	(2,329 – 8,512)	(9,893 – 22,980)
Tmax	4h	4h	4h	4h
AUC †	3,280	11,239	69,301	134,883
$t_{½}$ ‡	20.1	3.7	2.9	2.7

* Median (interquartile range, IQR), ng/ml or ng/g

† Area under the curve (AUC)$_{48h}$ for plasma (ng * h/ml); AUC$_{24h}$ for tissues (ng * h/g)

‡ Half-life ($t_{½}$, h)