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Pharmacokinetic profile of raltegravir, elvitegravir and dolutegravir in plasma and mucosal secretions in rhesus macaques

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Objectives: Pharmacokinetic studies in animal models are important for assessing the prophylactic potential of antiretroviral drugs for HIV prevention. This study sought to identify clinically relevant doses of the marketed integrase inhibitors raltegravir, elvitegravir and dolutegravir in macaques and investigate drug penetration and antiviral activity in mucosal secretions.

Methods: Macaques received one oral dose of raltegravir, elvitegravir or dolutegravir alone or in combination with emtricitabine and tenofovir disoproxil fumarate followed by drug level measurements in blood and rectal and vaginal secretions. Antiviral activity was investigated in TZM-bl cells exposed to $SHIV_{162p3}$ in the presence of rectal secretions collected from treated animals.

Results: Plasma drug concentrations with 50 mg/kg raltegravir or elvitegravir were within the range seen in humans receiving 400–800 mg of raltegravir or 800 mg of unboosted elvitegravir but lower than with 150 mg of elvitegravir boosted with cobicistat. AUC_{0-24} values for dolutegravir increased proportionally with the dose, with a calculated human-equivalent dose of 20 mg/kg. Elvitegravir showed the highest penetration in rectal and vaginal fluids despite the absence of pharmacological boosting, followed by raltegravir and dolutegravir. Rectal secretions collected at 24 h from treated macaques blocked infection of TZM-bl cells by 50% at dilutions of 1/1000 (raltegravir), 1/800 (dolutegravir) and >1/30000 (elvitegravir).

Conclusions: We defined macaque doses of HIV integrase inhibitors that recapitulate human clinical doses, which will facilitate efficacy and dose escalation studies in macaques. High and sustained drug concentrations and activity in mucosal secretions suggest that integrase inhibitors are promising candidates for HIV prevention.

Keywords: integrase inhibitors, non-human primates, antiviral activity

Introduction

Macaque models of simian immunodeficiency virus (SIV) or SHIV (an SIV/HIV chimera) transmission are widely used to evaluate the efficacy of antiretroviral drugs in preventing HIV infection, either as pre-exposure or post-exposure prophylaxis (PrEP and PEP, respectively).[1] Studies on SIV-exposed macaques receiving PEP with tenofovir showed that PEP was most effective when initiated soon after exposure and continued for 4 weeks, and helped define guidelines to manage occupational and non-occupational HIV exposures in humans.[2–4] Oral PrEP with emtricitabine and tenofovir disoproxil fumarate prevented rectal and vaginal SHIV infection in rhesus and pigtail macaques, and predicted the efficacy of emtricitabine/tenofovir disoproxil fumarate in humans.[5–9] However, human clinical trials with daily emtricitabine/tenofovir disoproxil fumarate in humans also highlighted the difficulty participants experienced adhering to the daily oral regimen as only ~50%–80% had consistently detectable tenofovir, a marker of compliance. Very low adherence (<30%) was the likely reason why two other studies (VOICE and FEM-PrEP) failed to show any efficacy of daily emtricitabine/tenofovir disoproxil fumarate.[10,11] New PrEP regimens that can be given peri-coitally and do not require daily dosing may potentially increase adherence and effectiveness of PrEP. Such on-demand emtricitabine/tenofovir disoproxil fumarate regimens have demonstrated efficacy in macaques and their acceptability and effectiveness are currently being evaluated in humans.[12,13]

The marketed HIV integrase inhibitors raltegravir, elvitegravir and dolutegravir are all important components of treatment regimens for HIV-1-infected persons but also are attractive

candidates for either on-demand or daily prophylaxis. All three drugs are well tolerated, very potent [protein-adjusted IC_{95} (PA-IC_{95}) ranging from 16 to 64 ng/mL], and bind tightly to pre-integration complexes with long (>7 h) disassociation half-lives.[14] As strand transfer inhibitors, these drugs block HIV integration into cellular DNA, a step that occurs after reverse transcription and >6 h after infection.[15] This unique mechanism of action may extend the coital dosing window of integrase inhibitors beyond what is afforded by reverse transcriptase inhibitors, and potentially provide more flexibility for oral dosing and extended protection from infection. Proof of concept for post-exposure protection by an integrase inhibitor was recently shown in macaques receiving a vaginal raltegravir gel 3 h after SHIV exposure.[15] In this study, five of six macaques were protected during 20 vaginal SHIV exposures.[15] These data heighten interest in oral raltegravir and other integrase inhibitors for HIV prevention.

Macaque models provide an invaluable tool to assess the prophylactic potential of oral integrase inhibitors through pharmacokinetic and subsequent efficacy studies. Pharmacokinetic assessments can provide information on systemic as well as rectal and vaginal drug distribution, the sites of early virus replication during sexual transmission. However, modelling clinically relevant doses in macaques requires information on the appropriate dosing that reproduces drug exposures observed in humans since macaques generally metabolize drugs differently from humans.[16] Of the three licensed integrase inhibitors, only raltegravir has been administered orally to macaques, at doses ranging between 20 and 100 mg/kg, mostly in combination with emtricitabine and tenofovir.[17,18] In one study, 50 mg/kg raltegravir given as monotherapy consistently reduced plasma viraemia. However, none of the studies evaluated systemic or mucosal raltegravir concentrations and how they relate to human levels. Likewise, drug distribution studies with oral elvitegravir or dolutegravir in macaques have not been done. Here we performed a single-dose pharmacokinetic study with raltegravir, elvitegravir and dolutegravir in rhesus macaques to identify doses that mimic human drug exposures. We also measured drug concentrations in rectal and vaginal secretions and related drug levels to antiviral activity. This study provides the basis for a rational selection of integrase inhibitors and doses for efficacy studies in macaques.

Materials and methods

Drug preparation and dosing

Raltegravir (Isentress) and dolutegravir (Tivicay) tablets were ground to powder and suspended in PBS. Elvitegravir was dissolved in a vehicle containing 65/20/15 PEG 400 (polyethylene glycol)/TPGS (tocopherol polyethylene glycol 1000 succinate)/10 nM phosphate buffer, pH 8. The final pH was adjusted to 7.

The initial doses of raltegravir, elvitegravir and dolutegravir were selected to approximate daily human doses of 800 mg of raltegravir, 800 mg of elvitegravir or 50 mg of dolutegravir. Doses were adjusted based on the differences in weight and BMI between macaques and humans.[19] We calculated human-equivalent doses of ~50 mg/kg raltegravir, 50 mg/kg elvitegravir and 3 mg/kg dolutegravir based on K_m values of 37 and 12 for humans and macaques, respectively. For drug pharmacokinetic studies, raltegravir, elvitegravir and dolutegravir were dosed with emtricitabine (20 mg/kg) and tenofovir disoproxil fumarate (22 mg/kg). These emtricitabine and tenofovir disoproxil fumarate doses recapitulate drug exposure achieved in humans treated with 200 mg of emtricitabine and 300 mg of tenofovir disoproxil fumarate.[5] Analyses of protein binding and drug activity were done in animals receiving raltegravir, elvitegravir or dolutegravir without emtricitabine/tenofovir disoproxil fumarate. Drugs were administered orally by gavage to groups of five macaques followed by collection of blood and rectal and vaginal secretions over time.

All animal procedures were performed according to NIH guidelines and approved by the Institutional Animal Care and Use Committee (IACUC) of the CDC. Macaques were housed at the CDC under the full care of CDC veterinarians in accordance with the standards incorporated in the Guide for the Care and Use of Laboratory Animals (National Research Council of the National Academies, 2010). All procedures were performed under anaesthesia using ketamine, and all efforts were made to minimize suffering, improve housing conditions and provide enrichment opportunities.

Analysis of drug concentrations in plasma, rectal secretions and vaginal secretions

The concentrations of raltegravir, elvitegravir and dolutegravir were measured by HPLC–MS/MS. Briefly, raltegravir and elvitegravir were extracted from 100 μL of plasma, 20 μL of secretion eluates or 20 μL of dialysis buffer by protein precipitation using 500 μL of methanol containing 25 ng of appropriate internal standard; deuterium-labelled raltegravir (RAL-d3) (Toronto Research Chemicals, Toronto, Canada) was used as the internal standard for raltegravir and deuterium-labelled elvitegravir (EVG-d6) (Toronto Research Chemicals, Toronto, Canada) was used as the internal standard for elvitegravir and dolutegravir. Dolutegravir in plasma (100 μL) was extracted the same way as raltegravir and elvitegravir, while dolutegravir in secretions or dialysis buffer was extracted from 20 μL of sample mixed with 80 μL of normal human plasma (Interstate Blood Bank, Memphis, TN, USA). After a brief centrifugation and removal of protein precipitates, the supernatant was evaporated to near dryness at room temperature in a biological cabinet, and re-suspended in 150 μL of mobile phase A (0.2% formic acid in water). Ten microlitres of the final solution was injected onto a 0.5×150 mm HALO Phenyl Hexyl column (ABSciex, Foster City, CA, USA) connected to an Ekspert MicroLC 200 HPLC system (ABSciex). An aqueous acetonitrile mobile-phase gradient was used to elute the analytes from the column and into a Model 3200 QTrap MS/MS system (ABSciex). Mass transitions (*m/z*) were monitored in positive multiple reaction monitoring (MRM) mode. Analyte concentrations were determined from a standard curve with a range of 1–2000 ng/mL using Analyst 6.1 software (ABSciex). The limit of quantification for all analytes was 10 ng/mL in plasma samples and 50 ng/mL in secretion eluates and dialysis buffer.

Rectal and vaginal secretions were collected in wicks (Weck-Cel Surgical Spear, Medtronic Ophthalmic, Jacksonville, FL, USA) using a modification of an established protocol used for the analysis of genital tract immunoglobulins and cytokines in human cervical secretions.[20] Briefly, dry wicks (four wicks/animal) were inserted 5 cm into the rectum or vaginal cavity of recumbent macaques and maintained for 5 min. Elution of elvitegravir and raltegravir from sponges was done by addition of 50 μL of elution buffer as described previously.[20] After 15 min on ice to allow the buffer to diffuse, each sponge was transferred to a PCR purification column (QIAquick PCR Purification Kit, Qiagen) and then centrifuged at 14000 rpm for 5 min at 4°C to remove particulate matter. The total eluates (geometric mean, 95% CI) obtained from four vaginal or rectal sponges were 129 (127–131) and 146 (134–159) μL, respectively. We used those values to calculate the total volume of rectal or vaginal secretion collected in one sponge after correction for the volume of elution buffer added to the sponges and the fraction of liquid retained in a sponge.[13] On average, a sponge contained 10 (10–11) and 13 (11–15) μL of vaginal or rectal secretions, respectively. These volumes were then used to calculate the amount of elvitegravir and raltegravir in nanograms per millilitre of secretion. Elution of dolutegravir from sponges was done directly with 250 μL of methanol per sponge since normal elution buffer interfered

with assay performance (not shown). The average volume of secretion contained in a sponge (10 or 13 μL, see above) was then used to calculate the concentration of dolutegravir per millilitre of secretion.

Pharmacokinetic parameters were estimated using non-compartmental methods (WinNonlin software version 5.2; Pharsight Corporation). C_{max} was determined visually. T_{max} was defined at C_{max}. AUC values were calculated over 24 or 48 h.

Analysis of protein binding

Protein binding was determined by rapid equilibrium dialysis as previously described (Rapid Equilibrium Dialysis Device System, Thermo Scientific).[21] Briefly, a 100 μL volume of plasma or rectal secretions eluted in PBS was incubated for 10 h at 37°C in dialysis cartridges followed by protein precipitation and measurement of total and free drug concentrations by HPLC–MS/MS.[21,22] These values were used to calculate the percentage of drug bound to proteins according to the manufacturer's instructions.

Antiviral activity of raltegravir, elvitegravir and dolutegravir in rectal secretions

The antiviral activity of raltegravir, elvitegravir and dolutegravir in rectal secretions was investigated by measuring the ability of the secretions to block infection of TZM-bl cells with $SHIV_{162p3}$ *in vitro*. Briefly, macaques ($n=5$ per drug) received a single oral dose of raltegravir (50 mg/kg), elvitegravir (50 mg/kg) or dolutegravir (30 mg/kg) without emtricitabine/tenofovir disoproxil fumarate followed by collection of rectal secretions at 5, 24 or 48 h. Secretions collected from five macaques at each timepoint were pooled, serially diluted and then added to triplicate wells containing 3×10^4 TZM-bl cells and $\sim10^7$ particles of $SHIV_{162p3}$ (based on genomic RNA levels). Luciferase activity (relative light units) was measured after 48 h of incubation at 37°C and used to quantify the number of infectious particles per sample. The percentage of cell protection was calculated by measuring the ratio between luciferase activities in the presence and absence of secretions. Rectal secretions collected from the same macaques prior to drug dosing were used as baseline controls.

Results

Pharmacokinetic profiles of raltegravir, elvitegravir and dolutegravir in blood

Figure 1 shows the pharmacokinetic profile of each drug in blood and Table 1 summarizes the pharmacokinetic parameters. With 50 mg/kg raltegravir, the AUC_{0-24} and C_{max} values [median (minimum–maximum)] were 3635 (1040–6752) ng·h/mL and 299 (129–924) ng/mL, respectively. In humans, 400 mg of raltegravir twice a day resulted in variable AUC_{0-24} and C_{max} values, ranging from 1700 to 7000 ng·h/mL and 500 to 2000 ng/mL, respectively, while 800 mg once a day resulted in similar or 2- to 4-fold higher AUC_{0-24} and C_{max} values.[23–25] Thus, the overall raltegravir exposure with 50 mg/kg as measured by AUC values was within the range of variability seen in humans receiving 400 mg twice a day or 800 mg once a day.

With 10 mg/kg elvitegravir, plasma AUC_{0-24} values [420 (269–1058) ng·h/mL] and C_{max} values [69 (25–226) ng/mL] were well below the values seen in humans receiving unboosted elvitegravir at 800 mg once a day or 200–800 mg twice a day.[26] With the 50 mg/kg dose, AUC_{0-24} increased to 4012 (829–9353) ng·h/mL, which is well within the range seen with 800 mg once a day or twice a day in humans (3570–5510 ng·h/mL)[26] However, C_{max} [282 (25–752) ng/mL] still remained lower than in humans (836–940 ng/mL).[26] Plasma elvitegravir concentrations at 24 h (C_{tau}) were undetectable with the 10 mg/kg dose and were 53 (32–61) ng/mL with the 50 mg/kg dose, in accordance with humans receiving 800 mg twice a day (48 ng/mL) (Figure 1 and Table 1).

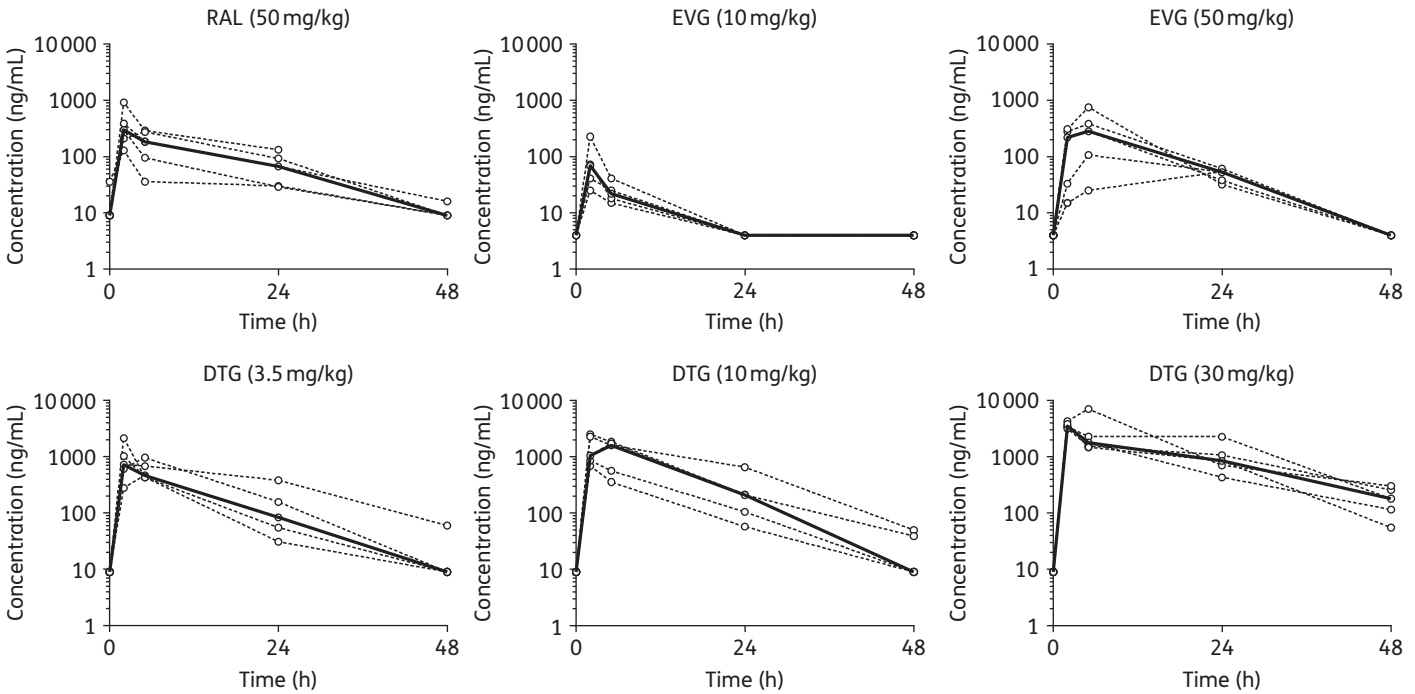

Figure 1. Pharmacokinetic profiles of raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG) in plasma from rhesus macaques. Macaques ($n=5$ per drug and dose) received a single oral drug dose followed by collection of plasma and drug level measurement by HPLC–MS/MS. Broken lines represent each individual macaque. The continuous line represents median drug concentrations.

Table 1. Pharmacokinetic parameters of raltegravir, elvitegravir and dolutegravir in rhesus macaques

Drug and dose	C_{max} (ng/mL)	C_{tau} (ng/mL)	T_{max} (h)	AUC_{0-24} (ng·h/mL)
Raltegravir				
50 mg/kg	299 (129–924)	67 (29–132)	2 (2–5)	3635 (1040–6752)
Elvitegravir				
10 mg/kg	69 (25–226)	BLQ	2	420 (269–1058)
50 mg/kg	282 (25–752)	53 (32–61)	5	4012 (829–9353)
Dolutegravir				
3.5 mg/kg	963 (466–2150)	97 (31–381)	2 (2–5)	10933 (7752–18263)
10 mg/kg	1719 (691–2498)	209 (57–553)	2 (2–5)	26505 (7002–37868)
30 mg/kg	3503 (3125–7055)	847 (433–2268)	2 (2–5)	50994 (39124–106952)

BLQ, below limit of quantification.
All values shown are median (minimum–maximum).

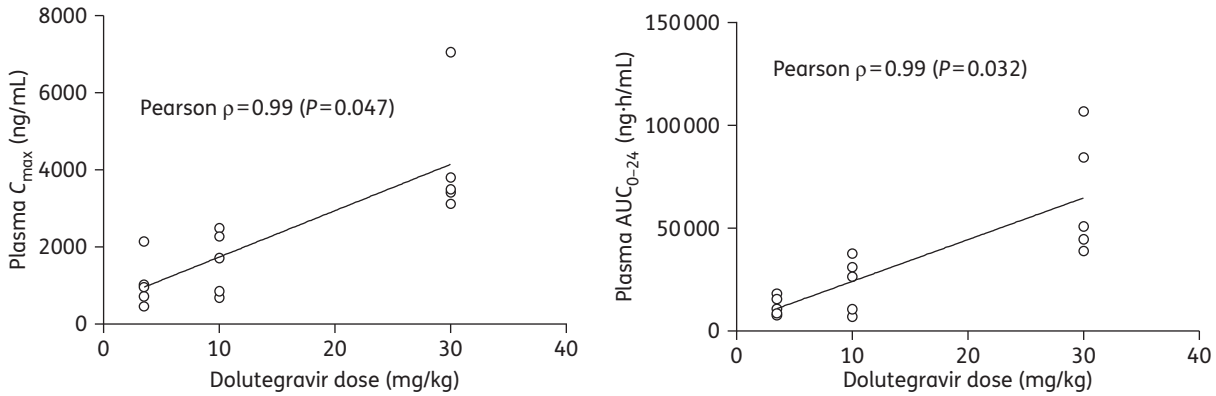

Figure 2. Dose proportionality between oral dolutegravir and plasma dolutegravir concentrations. Macaques ($n=5$ per drug and dose) received orally 3.5, 10 or 30 mg/kg dolutegravir followed by drug level measurements by HPLC–MS/MS. Dose–response relationship between oral dolutegravir dose and plasma C_{max} or plasma AUC_{0-24} is shown. Each symbol represents an individual macaque.

Dolutegravir was given at 3.5, 10 or 30 mg/kg. Table 1 and Figure 2 show that both C_{max} and AUC values increased proportionally within this dosing range. In humans, a single 50 mg dose of dolutegravir resulted in AUC_{0-24} and C_{max} values of 38000 ng·h/mL and 3000 ng/mL, respectively.[27] A dose–response analysis of C_{max} and AUC values showed that a macaque dolutegravir dose of ~20 mg/kg recapitulated drug exposure achieved with 50 mg of dolutegravir in humans (Figure 2).

We also evaluated raltegravir, elvitegravir and dolutegravir protein binding in plasma. As in humans, all three integrase inhibitors were highly bound to proteins; the percentage of drug bound (geometric mean, 95% CI) to plasma proteins was 91% (81%–101%) for raltegravir, 96.7% (93%–100%) for elvitegravir and 99% for dolutegravir. As expected, elvitegravir, raltegravir and dolutegravir did not affect the concentrations of emtricitabine or tenofovir in plasma (data not shown).

Raltegravir, elvitegravir and dolutegravir concentrations in rectal and vaginal secretions

We next measured drug concentrations in rectal and vaginal secretions after 50 mg/kg raltegravir, 50 mg/kg elvitegravir or 30 mg/kg dolutegravir. Figure 3 shows the concentrations achieved over time for each drug. In vaginal secretions (continuous line), peak concentrations (median, minimum–maximum) of raltegravir [9502 (1145–56795) ng/mL], elvitegravir [48296 (870–335750) ng/mL] and dolutegravir [261 (201–813) ng/mL] were achieved at 5 (2–24) h, 24 h and 5 (2–24) h, respectively. In rectal secretions (broken line), peak levels (13000 ng/mL for raltegravir, 98119 ng/mL for elvitegravir and 28915 ng/mL for dolutegravir) were also high and, in the cases of raltegravir and dolutegravir, were achieved later (24 h) than in vaginal secretions (Figure 3).

We next calculated the ratio between AUC_{0-24} values in rectal and vaginal secretions as a measure of relative tissue exposure to each integrase inhibitor (Table 2). The raltegravir AUC_{0-24} values were similar in rectal (127042 ng·h/mL) and vaginal (153618 ng·h/mL) secretions, while rectal AUC_{0-24} values for elvitegravir (2784000 ng·h/mL) were 6.1-fold higher than those seen in vaginal secretions (458870 ng·h/mL). Rectal AUC values for dolutegravir were similar to vaginal values with the 10 mg/kg dose and 40-fold higher than in vaginal secretions with the 30 mg/kg dose (Table 2).

We also calculated the ratio between AUC_{0-24} values in mucosal secretions and plasma as a measure of relative drug

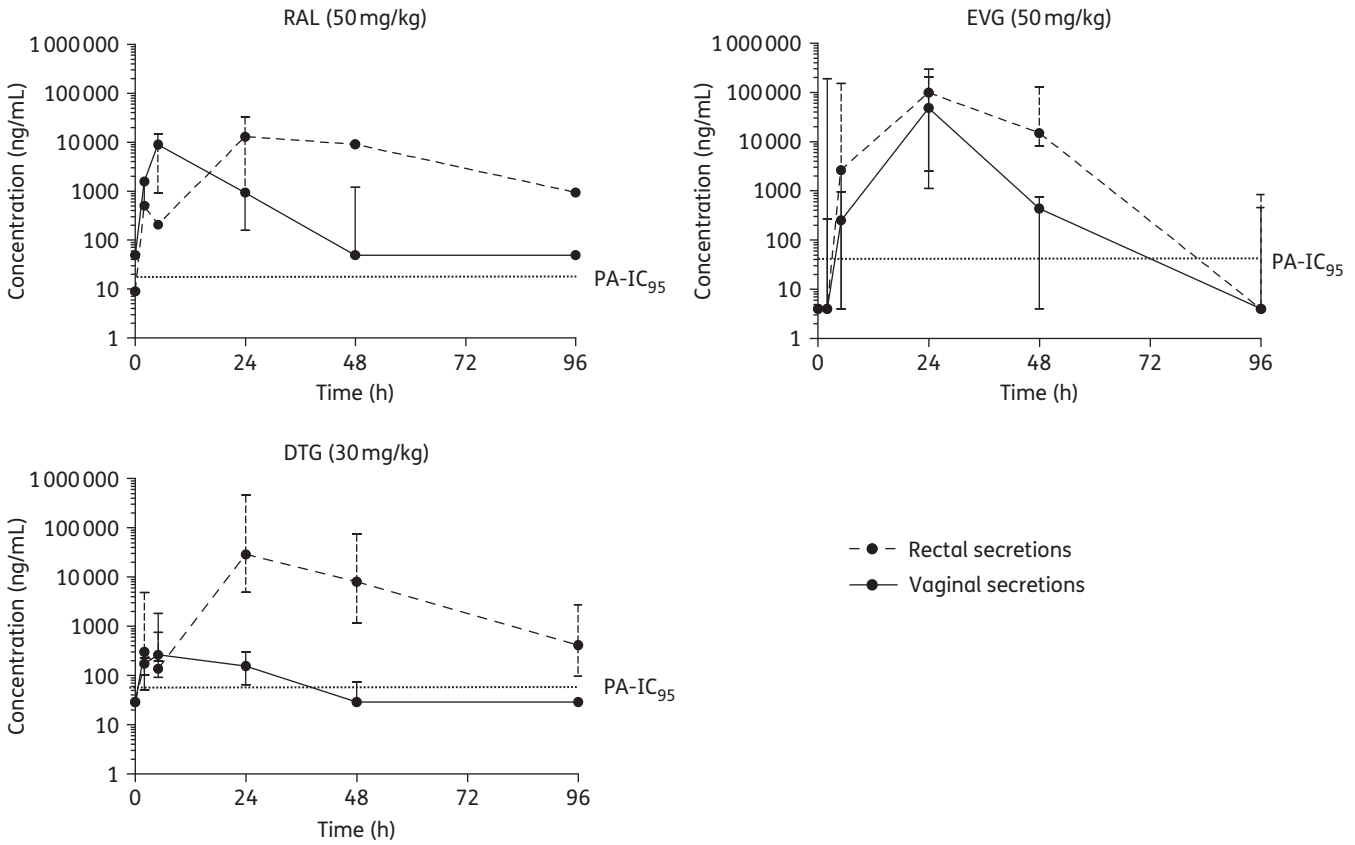

Figure 3. Pharmacokinetic profiles of raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG) in rectal and vaginal secretions. Macaques ($n=5$ per drug and dose) received a single dose of raltegravir (50 mg/kg), elvitegravir (50 mg/kg) or dolutegravir (30 mg/kg) followed by collection of rectal and vaginal secretions in wicks. Results are expressed as median concentrations measured by HPLC–MS/MS. Vertical lines denote the IQR. Horizontal lines indicate the PA-IC_{95} value for each drug.

Table 2. Relative penetration of raltegravir, elvitegravir and dolutegravir in rectal and vaginal secretions

	AUC_{0-24} (ng·h/mL)[a]			AUC_{0-24} ratio		
	rectal secretions (RS)	vaginal secretions (VS)	blood plasma (BP)	RS:VS	RS:BP	VS:BP
Raltegravir						
50 mg/kg	127042	153618 (12479–718990)	3635 (1040–6752)	0.8	35	42
Elvitegravir						
50 mg/kg	2784000 (9121–4236000)	458870 (11172–3190000)	4012 (829–9353)	6.1	694	114
Dolutegravir						
10 mg/kg	6085 (3063–16636)	7281 (1390–13496)	26505 (7002–37868)	0.84	0.23	0.27
30 mg/kg	275968 (16708–7342000)	6862 (3026–10477)	50994 (39124–106952)	40	5.4	0.13

The value for raltegravir in rectal secretions represents a single measurement of pooled secretions prepared from five macaques at baseline, 2 h, 5 h, 24 h or 48 h.
[a]All values are median (minimum–maximum).

penetration. Table 2 shows that overall raltegravir concentrations in rectal and vaginal secretions were consistently higher than in plasma (35- and 42-fold, respectively). With unboosted elvitegravir, relative concentrations were also higher at the two mucosal sites (694- and 114-fold, respectively). In contrast, dolutegravir penetration in rectal and vaginal secretions was low with the 10 mg/kg dose (0.23 and 0.27 times that in blood plasma, respectively). Vaginal dolutegravir penetration remained low (0.13 times that in blood plasma) with 30 mg/kg dolutegravir while rectal penetration with this high dolutegravir dose was

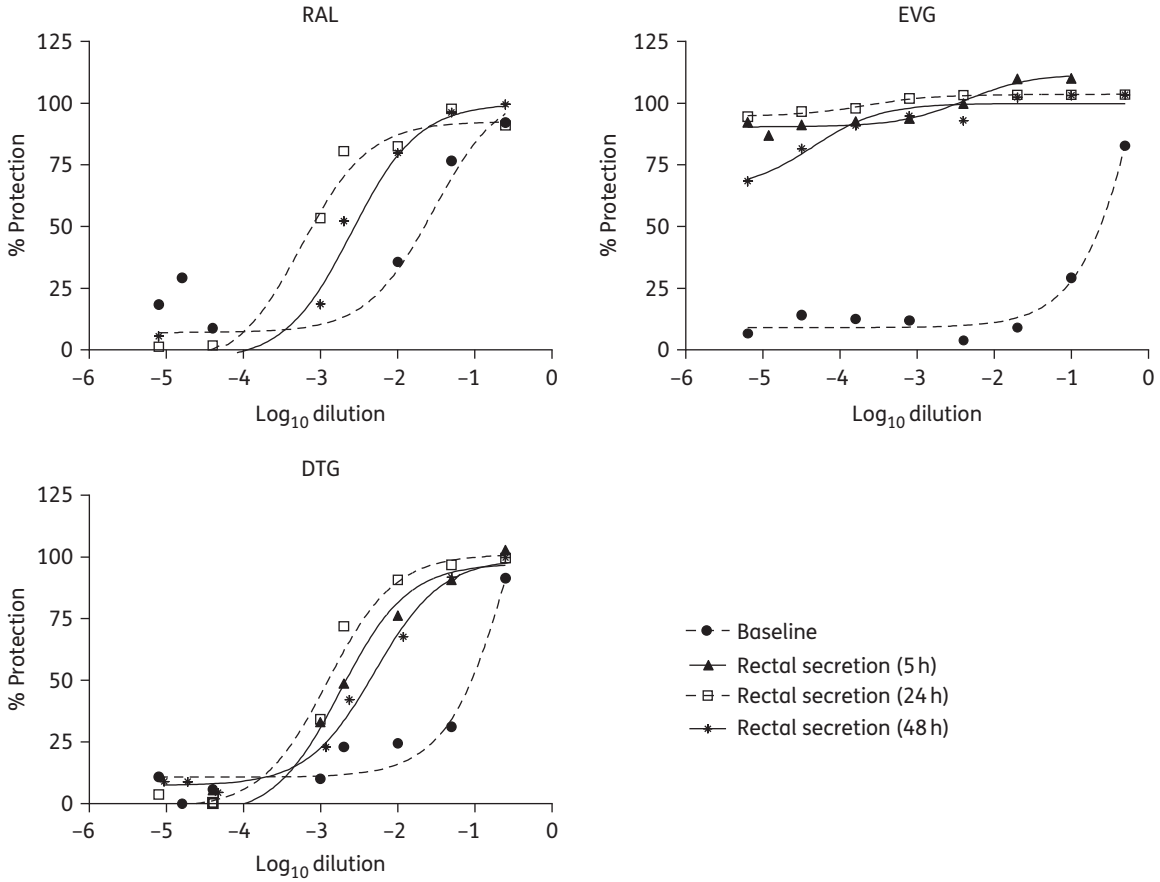

Figure 4. Antiviral activity of raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG) in rectal secretions. Macaques ($n=5$ per drug) received a single oral dose of raltegravir (50 mg/kg), elvitegravir (50 mg/kg) or dolutegravir (30 mg/kg) followed by collection of rectal secretions at 5, 24 or 48 h. Secretions from each timepoint were pooled and serial dilutions were evaluated for their ability to prevent infection of TZM-bl cells with $SHIV_{162p3}$. Data represent the percentage of cell protection relative to the dilution. Baseline secretions collected from the same macaques prior to drug dosing were used as controls.

5.4-fold higher than in blood plasma (Table 2). These results show that raltegravir, elvitegravir and dolutegravir penetrate with different efficiencies into rectal and vaginal tissues.

Antiviral activity of raltegravir, elvitegravir and dolutegravir in rectal secretions

We next investigated the antiviral activity of raltegravir, elvitegravir and dolutegravir in secretions. We focused on rectal secretions since drug concentrations were generally higher than in vaginal secretions and were also sustained for longer periods of time. For this analysis we dosed groups of five macaques with raltegravir, elvitegravir or dolutegravir and then evaluated the ability of secretions to block infection of TZM-bl cells with SHIV *in vitro*. We first defined background activity in secretions collected prior to drug dosing. On average, a 1/10 dilution (range 1/25–1/3) of secretions from untreated animals was able to block infection of TZM-bl cells by 50% (EC_{50}). After raltegravir dosing, the EC_{50} was achieved at a 1/1000 (24 h) or a 1/350 dilution (48 h) (Figure 4). Similar results were found with dolutegravir; EC_{50} values were obtained at a 1/400 dilution at 5 h, a 1/800 dilution at 24 h and a 1/250 dilution at 48 h. Secretions collected from macaques receiving elvitegravir protected TZM-bl cells by >90% even at a 1/31 000 dilution. These results demonstrate high and sustained antiviral activity of raltegravir, elvitegravir and dolutegravir in rectal secretions.

We also examined changes in antiviral activity of rectal secretions over time by comparing EC_{50} values achieved with secretions with those seen with drug spiked in PBS or in blank secretions collected from untreated animals. Figure 5 shows that the EC_{50} value achieved with secretions collected 5 h after dolutegravir treatment (0.9 nM; 95% CI 0.4–2.0) was similar to that seen with dolutegravir spiked in PBS (1.2 nM; 95% CI 0.6–2.6). In contrast, the EC_{50} values observed with secretions collected at 24 h (51.1 nM; 95% CI 34.2–76.2) or 48 h (75.3 nM; 95% CI 40.9–138.8) were 42- to 63-fold higher than that seen with spiked dolutegravir, and similar to the protein-adjusted EC_{50} value of 38 nM.[28] Overall, these findings demonstrate that all dolutegravir present in secretions collected at 5 h was active but only a fraction of drug detected at 24–48 h retained antiviral activity. We could not collect secretions at 5 h after raltegravir dosing, although the analysis of inhibition curves at 24 h or 48 h also suggested a reduction in activity relative to raltegravir spiked in PBS (Figure 5). A similar analysis with elvitegravir showed high activity with

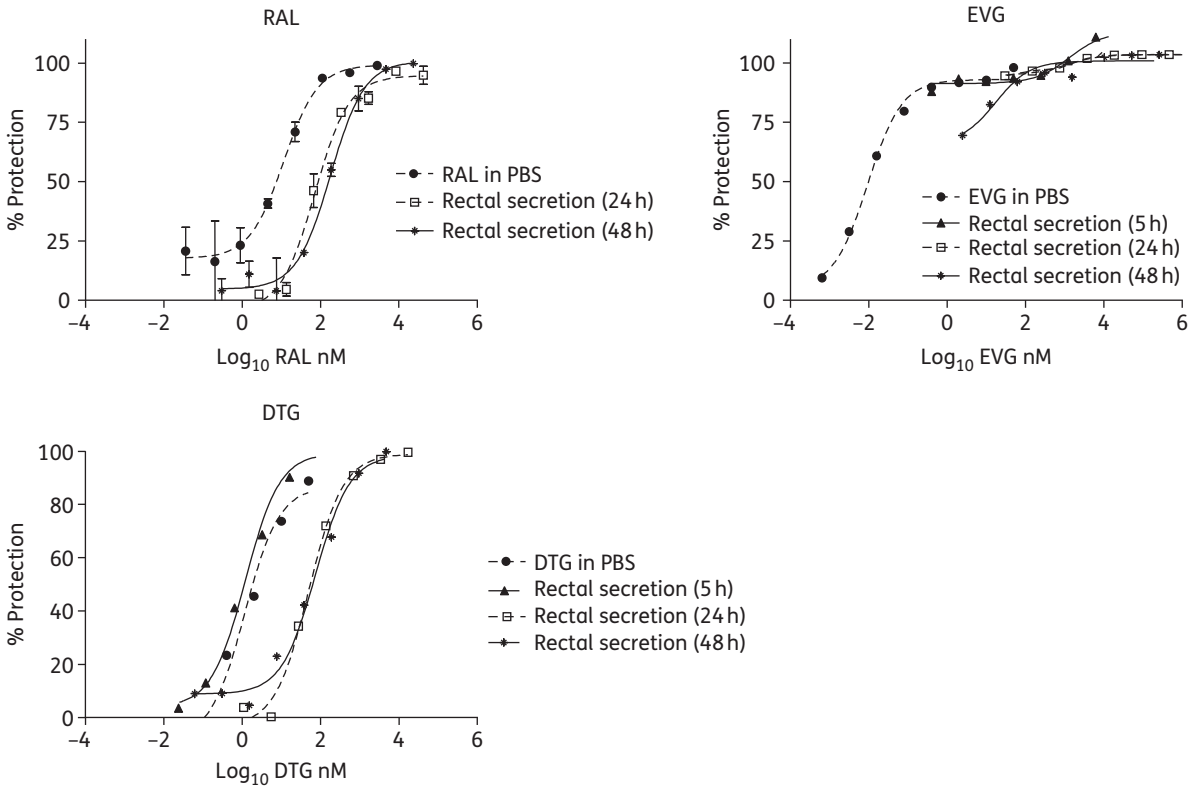

Figure 5. Inhibition curves with raltegravir (RAL), elvitegravir (EVG) and dolutegravir (DTG) from rectal secretions. Rectal secretions collected from treated macaques at 5, 24 or 48 h were evaluated for their ability to bock infection of TZM-bl cells with $SHIV_{162p3}$. Data represent the percentage of cell protection as a function of the concentration of drug measured in secretions by HPLC–MS/MS. Inhibition curves obtained with drug spiked in PBS are shown as reference.

secretions collected at 5 or 24 h and an apparent reduction in activity at 48 h (EC_{50} values were outside the linear range) (Figure 5). Overall, while all rectal secretions exhibited antiviral activity *in vitro*, we observed a trend towards lower activity relative to free drug over time. Possible mechanisms that can contribute to such changes include variable non-specific activity in rectal secretions and different degrees of protein binding of drugs.

Discussion

We investigated the pharmacokinetic profiles of raltegravir, elvitegravir and dolutegravir in macaques and identified oral doses that recapitulate systemic drug concentrations achieved in humans receiving therapeutic doses. As in humans, all three drugs were highly bound to plasma proteins and differed in their ability to penetrate mucosal tissues. Dolutegravir levels in rectal and vaginal secretions were only 23% and 27% of those seen in plasma (at a dose of 10 mg/kg), although rectal concentrations were higher with a higher dolutegravir dose (30 mg/kg). In contrast, raltegravir concentrations in rectal secretions were 35-fold higher than in plasma. These observations are in line with first-dose kinetic studies in humans, which also noted low mucosal dolutegravir concentrations and high raltegravir penetration.[27,29–31] Since high drug penetration into anatomical sites permissive of early HIV replication during sexual transmission is likely important to prevent HIV infection, our observations suggest that raltegravir may have an added advantage over dolutegravir for PrEP or PEP. Future macaque virus challenge studies may determine whether therapeutic or possibly lower doses of dolutegravir or raltegravir prevent infection.[32]

In humans, co-administration of elvitegravir with inhibitors of cytochrome P450 CYP3A enzymes such as ritonavir or cobicistat boosts systemic elvitegravir levels by ∼20-fold.[26,33] Since our study was done without a pharmacoenhancer, the plasma drug concentrations seen with the 50 mg/kg dose were only reflective of those achieved with unboosted elvitegravir in humans.[26] Notably, mucosal elvitegravir levels were very high despite low systemic elvitegravir levels, as vaginal and rectal concentrations were 100- to 700-fold higher than plasma levels and remained well above the PA-IC_{95} for up to 48 h. High mucosal drug penetration despite high plasma protein binding has also been noted for other drugs, such as maraviroc.[34,35] As data on mucosal elvitegravir penetration become available in humans, it will be important to see whether the concentrations of elvitegravir in rectal and vaginal fluids are also high and sustained and whether they are increased further by cobicistat.

As in humans, systemic dolutegravir concentrations in macaques increased in a dose-proportional manner.[36] We found that 20 mg/kg dolutegravir in macaques recapitulated systemic drug concentrations achieved with 50 mg in humans. However, the 20 mg/kg dose was much higher than the predicted 3 mg/kg macaque dose based on dose translation estimates using

human and macaque K_m factors.[19] This difference might be due to reduced dolutegravir absorption in macaques, or the administration of dolutegravir to fasting animals; in fasting humans, dolutegravir absorption can be reduced by up to 66%.[37] The dolutegravir dose identified in this study will likely result in maximal antiviral activity and also benefits modelling treatment and cure strategies in macaques, which have previously used a lower dolutegravir dose.[32]

Several observations can be made from the analysis of drug penetration in rectal secretions. First, all three integrase inhibitors achieved levels that were well above the PA-IC_{95} for up to 48 h. High drug concentrations were also associated with potent and sustained antiviral activity, demonstrating that a significant amount of drug in rectal secretions remained active. Interestingly, dolutegravir in secretions collected at 5 h had similar antiviral activity to dolutegravir spiked in PBS, suggesting that most of the drug was unbound to proteins. In contrast, dolutegravir at 24–48 h was less active and showed EC_{90} estimates close to the protein-adjusted values.[35] It is possible that mucosal protein binding at 5 h had not yet reached equilibrium, thus resulting in more free dolutegravir and greater antiviral effect.

Of the three integrase inhibitors, elvitegravir showed the highest penetration in rectal and vaginal fluids despite the absence of pharmacological boosting. The concentrations of elvitegravir in rectal secretions were also ~6-fold higher than in vaginal secretions. It is not clear whether faecal elimination of elvitegravir and mucus trapping contributed to the high rectal elvitegravir concentrations since ~95% of the oral elvitegravir dose is excreted in faeces compared with 50% for raltegravir and dolutegravir.[38,39] However, elvitegravir concentrations in vaginal fluids were also the highest among the three drugs, suggesting that drug distribution from blood plasma is a primary mechanism for elvitegravir accumulation in mucosal tissues. If confirmed in humans, the finding of high elvitegravir concentrations and antiviral activity in rectal and vaginal secretions is very promising for HIV prevention particularly for coital dosing, since these are the two primary sites of HIV infection. The high mucosal elvitegravir level in the absence of boosting is also promising as it raises the possibility of using unboosted elvitegravir, which can reduce the cost and the risk of drug–drug interactions. The data support challenge studies in macaques to assess whether unboosted elvitegravir is efficacious.

Our study is an important first step towards evaluating the prophylactic potential of these drugs in macaque models of HIV infection. The same approach with emtricitabine and tenofovir disoproxil fumarate provided proof of concept for efficacy in humans, and demonstrated that protection in macaques and humans was achieved at similar intracellular tenofovir diphosphate concentrations.[5,6,13,40,41] However, our study has some limitations. First, we did not model boosted elvitegravir since macaque doses of CYP3A4 inhibitors are not yet defined. It will be important to evaluate whether cobicistat or ritonavir can boost plasma elvitegravir concentrations to levels achieved in humans. Second, we did not directly measure drug concentrations in rectal or vaginal tissues. While mucosal dolutegravir and raltegravir levels in humans correlate with tissue drug concentrations, it is important to confirm whether the same correlation also occurs in macaques.[29,30] The comparison between mucosal drug levels in macaques and humans may be susceptible to differences in sample collection protocols and drug normalization methods (weight or volume).[29] Additional efforts should thus be focused on standardizing mucosal sampling procedures in macaques and humans. These studies may also help to identify surrogates of protection.

In summary, we defined the pharmacokinetic profiles of the three FDA-approved HIV integrase inhibitors in macaques and identified clinically relevant doses based on plasma drug levels. All three drugs showed high and sustained mucosal drug levels, suggesting that they may represent good candidates for PrEP or PEP. The data support efficacy studies with dose escalation designs in validated SHIV virus challenge experiments to define the prophylactic potential of this drug class and identify the minimal drug dose required for prevention.

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Transparency declarations

Authors J. G. G.-L. and W. H. are named in a US Government patent application related to methods for HIV prophylaxis. All other authors report no potential conflicts.

Disclaimer

The use of trade names is for information purposes only and does not imply endorsement by the Centers for Disease Control and Prevention. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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