Original article

Single and multiple dose pharmacokinetics of dolutegravir in the genital tract of HIV-negative women

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Background: Antiretrovirals that achieve adequate concentrations in anatomical sites of transmission are of interest for HIV prevention. A Phase I open-label pharmacokinetic (PK) study was performed to describe first dose (PK1) and steady-state (PK2) PKs of the integrase inhibitor dolutegravir (DTG) in blood plasma (BP), cervicovaginal fluid (CVF), cervical tissue (CT) and vaginal tissue (VT) in HIV type-1-negative women.

Methods: A total of 8 healthy females given DTG 50 mg daily for 5–7 days had 11 paired BP and CVF samples collected over 24 h following the first dose (PK1) and multiple dosing (PK2). Each woman underwent CT and VT biopsies at 1/4 time points at PK1 and PK2 to generate composite PK profiles. DTG concentrations were analysed by validated liquid chromatography-tandem mass spectrometry methods. Non-compartmental PK analysis was performed and Spearman rank correlations determined between matrices.

Results: BP areas under the concentration–time curve (AUCs) were similar to previous reports and concentrations remained greater than the protein-adjusted (PA) 90% inhibitory concentration (IC_{90}) for wild-type HIV (64 ng/ml). CVF exposures were approximately 6% of BP with low inter-individual variability. CT and VT exposures were 7% of BP at PK1, and 9–10% of BP at PK2 with 94% of samples >PA-IC_{90}. CT and VT concentrations were correlated to each other (ρ=0.70, *P*=0.003), and to CVF at steady state (ρ=0.52, *P*=0.04). Accumulation of DTG from PK1 to PK2 occurred in BP, CT and VT, but only marginally in CVF.

Conclusions: DTG BP PK were consistent with previously published values. CVF, CT and VT exposures were highly correlated. At PK2, DTG accumulated to a greater extent in tissue than in BP or CVF, suggesting increased tissue affinity.

Introduction

GSK1349572 (dolutegravir [DTG]) is an integrase strand transfer inhibitor (INSTI) currently in clinical development. It has a 14-h plasma half-life ($t_{½}$), which allows for once daily dosing without the need for pharmacokinetic (PK) boosting. In SPRING-1, a Phase IIb dose-ranging study in HIV-infected patients, 88–92% of patients dosed once daily with 10, 25 and 50 mg of DTG had undetectable HIV RNA at 48 weeks, compared with 82% of those taking the non-nucleoside reverse transcriptase inhibitor efavirenz (EFV) based on the intention-to-treat (ITT) exposed population [1]. These subjects also received either abacavir (ABC)/lamivudine (3TC) or tenofovir disoproxil fumarate (TDF)/emtricitabine (FTC). Two Phase III studies have evaluated the use of DTG in treatment-naive patients. In SPRING-2, a double-blind active-control non-inferiority study, DTG 50 mg daily was compared to the first integrase inhibitor to market, raltegravir (RAL) 400 mg twice daily, each given with either ABC/3TC or TDF/FTC [2]. Using an FDA snapshot analysis of the ITT exposed population, at 48 weeks, 88% of the patients receiving DTG and 85% of those receiving RAL had HIV RNA suppressed to <50 copies/ml. In SINGLE, a double-blind double-dummy non-inferiority study, DTG given with ABC/3TC was compared to EFV given as the fixed-dose combination with TDF/FTC [3]. Also using an FDA snapshot analysis of the ITT exposed population, at 48 weeks, 88% of DTG recipients and 81% of EFV

recipients had HIV RNA suppressed to <50 copies/ml primarily due to the higher discontinuation rates in the EFV arm. Additionally, DTG has been shown to maintain efficacy in RAL-experienced patients when given twice daily in an open-label cohort study [4]. Based on the promising results of these studies, DTG is a welcome addition to currently available antiretrovirals.

The purpose of this study is to describe first dose and steady-state PKs of DTG in cervicovaginal fluid (CVF), vaginal tissue (VT) and cervical tissue (CT) compared to blood plasma (BP) in HIV type-1-negative women. Although current antiretroviral therapy regimens decrease blood plasma HIV RNA to <50 copies/ml in a majority of patients, the risk of HIV type-1 transmission remains, as viral shedding in the female genital tract may be incompletely suppressed [5,6]. To eradicate replication-competent virus at the site of transmission, it is hypothesized that adequate local concentrations of antiretroviral drugs must be achieved [7]. Understanding PK behaviour of DTG in multiple female biological compartments will inform its role in preventing viral replication in the genital tract in HIV-infected women (particularly during acute HIV infection and in late-presenting pregnancy), and its potential in protecting mucosal surfaces against HIV infection for applications such as treatment as prevention, and post-exposure prophylaxis. This is the first study of female genital tract antiretroviral PKs to be completed prior to market approval.

Methods

Study design and population

The study was approved by the University of North Carolina Biomedical Institutional Review Board and performed under FDA Investigational New Drug application number 112,854. The trial was registered on clinicaltrials.gov under the identifier NCT01404806. All study activities were carried out in accordance with the ethical standards of the International Conference on Harmonization E6 Good Clinical Practice guidance. Informed consent was obtained from all participants prior to any study activities.

The women underwent screening within 45 days prior to enrolment. Screening procedures consisted of a complete medical history and physical examination: 12-lead electrocardiogram with cardiology interpretation and comprehensive laboratory studies (complete blood count with differential, liver function tests, serum chemistries, urinalysis and urine toxicology). Subjects were screened for active hepatitis B and hepatitis C, HIV, and other sexually transmitted infections such as syphilis, gonorrhea, trichomonas and chlamydia. Women underwent screening prior to study enrolment and DTG dosing. Subjects were eligible to participate if they were females with regular menstrual cycles between 18–35 years of age, inclusive at the date of screening, and had a body mass index (BMI) 18–30 kg/m^2 with total body weight >50 kg. Subjects were required to have fully intact genital and gastrointestinal tracts, with documentation of a normal Pap smear within 1 year. Subjects were excluded for any clinically significant abnormal lab value, physical examination finding or clinical condition that would interfere with study activities. Subjects were required to stop all prescription and non-prescription medications 7 days before and herbal supplements 14 days before study enrolment, and medications could not be restarted until study completion. Subjects were limited to consumption of <14 alcoholic drinks per week (1 drink =5 ounces [150 ml] of wine or 12 ounces [360 ml] of beer or 1.5 ounces [45 ml] of spirits), and acetaminophen at doses of <1 g/day.

All subjects had to use ≥1 acceptable form of contraception, including abstinence, bilateral tubal ligation, condoms with spermicide or foam, stable male partner with vasectomy or female only partners, or oral hormonal contraception started ≥3 months prior to enrolment. Subjects agreed to remain abstinent from all sexual activity and insertion of all vaginal products starting 72 h prior to enrolment until completion of all study activities. While taking DTG, the women were assessed for adverse events and medication adherence daily in person or via telephone and using a standardized head-to-toe assessment form. Vital signs, serum chemistries, complete blood count and urine pregnancy test were performed at each visit. All adverse events were graded by the study physician assistant and study physician using the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events.

Sample collection and processing

Subjects were admitted to the North Carolina Translational and Clinical Sciences (NC TraCS) Institute Clinical and Translational Research Center on day 1 (first dose) and either day 5, 6 or 7 of dosing with DTG 50 mg daily. Steady-state conditions were expected to be achieved within 5 days of dosing. Enrolment was scheduled 5–10 days after the end of the subject's menses. A witnessed DTG dose was given after an 8 h fast and standard research meals were provided throughout each 24 h inpatient sampling period. BP was collected in 3 ml K_2EDTA tubes (BD Diagnostics, Franklin Lakes, NJ, USA) pre-dose, then at 0.5, 1, 2, 3, 4, 6, 8, 12, 18 and 24 h following each dose. At those same time points, CVF was self-collected by the women using a volumetric aspiration device. Additionally, BP and CVF samples were collected at 48 and 72 h following the final dose. Immediately following collection, the CVF was transferred from the vaginal aspirator to a 2 ml cryovial and frozen at -80°C. Within 1 h after blood collection, K_2EDTA tubes stored on ice were processed

by centrifugation at 800×*g* at 4°C for 10 min. The resultant BP was then transferred to a 2 ml cryovial and frozen at -80°C.

VTs and CTs were collected by biopsy at one time point during each PK visit. Two women were assigned to each of four time points (either 3, 6, 12 or 24 h post-dose). Briefly, subjects were placed in the dorsal lithotomy position. After speculum insertion, the vaginal fornices and the entire cervix were anesthetized with topical 20% benzocaine spray (HurriCaine™, Beutlich Pharmaceuticals, Waukegan, IL, USA) and Lidocaine Hydrochloride Oral Topical Solution 2% (Boehringer Ingelheim, Roxane Laboratories, Inc., Columbus, OH, USA). Vaginal tissue biopsies were obtained at either the left or right vaginal fornix. Cervical biopsies were obtained at either the 3 or 9 o'clock position. Biopsies at the second time point were obtained at the opposite location. Approximately 4 mm×2 mm×2 mm specimen with a median (range) weight of 0.014 g (0.007–0.066), was obtained at both the vaginal and cervical sites using Baby Tischler forceps (Cooper Surgical, Trumbull, CT, USA). Samples were placed in separate labelled screw-capped polypropylene tubes, immediately snap-frozen in liquid nitrogen, and stored at -80°C until analysis. In order to be considered evaluable, subjects had to provide tissue and 80% of BP and CVF samples, including the pre-dose sample and 24-h sample at each PK visit.

Laboratory analyses

Quantification of DTG plasma concentrations was performed by protein precipitation and liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Calibration curves were obtained using a 1/concentration^2 weighted linear regression of analyte:internal standard peak area ratio versus nominal concentration. Compilation of concentration results and descriptive statistical analyses were performed using SCIEX Analyst version 1.6.1 (AB SCIEX, Foster City, CA, USA). An aliquot of 30 µl of each stored plasma sample was mixed with 600 µl of acetonitrile containing the isotopically-labelled internal standard, dolutegravir-$d_7{}^{15}$N (DTG-IS). Following vortex and centrifugation, a portion of the supernatant was diluted with 50:50 methanol:water prior to LC-MS/MS analysis. DTG was eluted from a Varian (Agilent) Pursuit Diphenyl (2.1×50 mm, 3 µm particle size) analytical column (Agilent Technologies, Santa Clara, CA, USA). An API-5000 triple quadrupole mass spectrometer (AB Sciex, Foster City, CA, USA) was used to detect the analyte. For DTG, the precursor ion was 420 m/z and the product ion was 277 m/z. For DTG-IS, the precursor ion was 428 m/z and the product ion was 283 m/z. Data were collected using AB Sciex Analyst Chromatography Software. The dynamic range of the assay was 20–20,000 ng/ml. All calibrators and quality control (QC) samples were within 15% of the nominal value for both within-day and between-day runs. The high, medium and low QC values used for plasma were 60.0, 600 and 16,000 ng/ml. Within-day and between-day precision calculations were <15%. The recovery range for DTG in plasma was 98.0–103%, and the recovery of DTG-IS was 104%.

The extraction of DTG from CVF samples required a fivefold dilution with 0.9% sodium chloride solution prior to analysis to lower the viscosity of the CVF. This dilution allowed for proper vortexing of the sample and facilitated quantitative transfer of the sample to the extraction tube. Calibration standards and QC samples were prepared in fivefold diluted human CVF. The lower viscosity of the diluted CVF also allowed for accurate preparation of standards and QCs. Since samples were diluted following collection, a 5× dilution factor was applied to all samples. An aliquot of 30 µl of each diluted CVF sample was mixed with 270 µl of acetonitrile containing DTG-IS. Following vortex and centrifugation, a portion of the supernatant was diluted with 50:50 methanol:water. DTG was detected using identical conditions to those described for plasma. The dynamic range of the assay was 1–1,000 ng/ml. The recovery range for DTG in CVF was 99.4–105%, and the recovery of DTG-IS was 101%. All calibrators and QC samples were within 15% of the nominal value. The low, medium and high QC values used for CVF were 3.00, 30.0 and 800 ng/ml. Within-day and between-day precision was <15%.

In order to extract DTG from VT and CT samples, the tissue samples were initially homogenized in 1 ml of 80:20 water:acetonitrile. An aliquot of 50 µl of the resulting homogenate was extracted by protein precipitation with acetonitrile containing DTG-IS. Following vortex and centrifugation, a portion of the supernatant was diluted with water. DTG was detected on an LC-MS/MS system using identical conditions as described above. During method validation, calibration standards were prepared in human VT homogenate. QC samples were prepared in human vaginal and CT homogenates. Method validation results indicated that calibration standards prepared in VT homogenate could be used successfully to quantitate DTG in both tissues. For sample analysis, calibration standards and QC samples were prepared in human VT homogenate. The dynamic range of the assay was 0.2–200 ng/ml homogenate. The recovery range of DTG in vaginal homogenate was 50.7–63.4%, and the recovery of DTG-IS was 72.0%. The recovery range of DTG in cervical homogenate was 48.3–65.9% and the recovery of DTG-IS was 71.4%. All calibrators and QC samples were within 15% of the nominal value with precision values <15%. The low, medium and high QC values used for tissues were 0.600, 6.00 and 160 ng/ml.

Pharmacokinetic and statistical analyses

No formal sample size calculation was performed for this study. The sample size was chosen to generate PK data adequate for understanding penetration of DTG into the female genital tract considering the number sufficient to describe inter-patient variability and prior similarly performed PK studies. Non-compartmental analysis was performed using Phoenix WinNonlin version 6.3 (Certara LP, St. Louis, MO, USA). Maximum concentration (C_{max}) and time to maximum concentration (T_{max}) were determined by visualization of the concentration–time curves, and the area under the concentration–time curve over 24 h ($AUC_{0–24\,h}$) was determined using the trapezoidal rule (linear up/log down interpolation). BP and CVF half-life ($t_{½}$) was determined by concentrations collected 24–72 h after the final dose. Composite AUCs were determined following the first dose (PK1) and at steady state (PK2) for CT and VT using the geometric mean of the two concentrations from each woman at each of the four biopsy time points. Summary statistics and Spearman rank correlations between BP and CVF, CVF and GT, and CT and VT, were calculated using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). PK parameters are separated by day and by matrix and PK graphs were made using SigmaPlot 12.0 (Systat Software, Inc., Chicago, IL, USA). To calculate tissue penetration ratios, tissue VT and CT density was assumed to be 1.05 g/ml. Concentrations below the limit of detection were imputed as 0 and concentrations below the limit of quantification were imputed as half the lower limit of quantification for the particular matrix (the lowest point on the standard curve). Accumulation ratios were calculated between PK1 to PK2. Data are presented as median (25th–75th percentile) unless otherwise noted.

Results

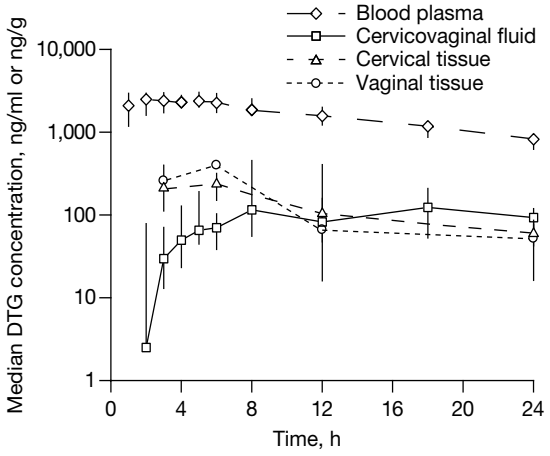
Demographics

A total of 11 women were enrolled to reach the target total of 8 women completing PK analysis for both PK1 and PK2. Three women dropped out during PK1 due to an inability to collect ≥80% of the required CVF samples. These three women are included in the adverse event data only. The median age of the eight evaluable women was 21 years (range 18–27). The median BMI was 22.5 kg/m^2 (range 21.0–29.2). All were Caucasian. The median PK profiles for BP and CVF, as well as the composite PK profiles for CT and VT are shown for PK1 (Figure 1) and for PK2 (Figure 2). The PK parameters for all matrices at both PK1 and PK2 are summarized (Table 1).

Plasma concentrations

C_{max} and T_{max} ranged from 1,350–4,380 ng/ml at 1–6 h after the first dose and 2,530–5,590 ng/ml at 1–4 h after multiple dosing. The median (IQR) $AUC_{0–24\,h}$ for PK1 was 38,300 ng•h/ml (31,600–45,400) and for PK2 was 55,400 ng•h/ml (43,300–62,200). The median (IQR) $t_{½}$ calculated using concentrations from 24–72 h post-dose was 14.8 h (13.6–16.1). The median (IQR) accumulation ratio of DTG in BP over 7 days of dosing was 1.42 (1.28–1.50).

Figure 1. Concentrations of dolutegravir in blood plasma, cervicovaginal fluid, cervical tissue and vaginal tissue following a single dose



Following the first dose of dolutegravir (DTG), the median (IQR) DTG concentrations at each time point are plotted for blood plasma (BP) and cervicovaginal fluid (CVF) and the median (range) DTG concentrations of the two individual subject cervical tissue (CT) and vaginal tissue (VT) samples at each time point are plotted as a composite pharmacokinetic curve. Error bars represent IQR for BP and CVF, and the two individual samples at each time point for CT and VT. The area under the plasma concentration–time curve (AUC) ratio of CVF to BP is 0.07 (IQR 0.05–0.18) following the first dose and the AUC ratio of tissue to CVF is 1.05.

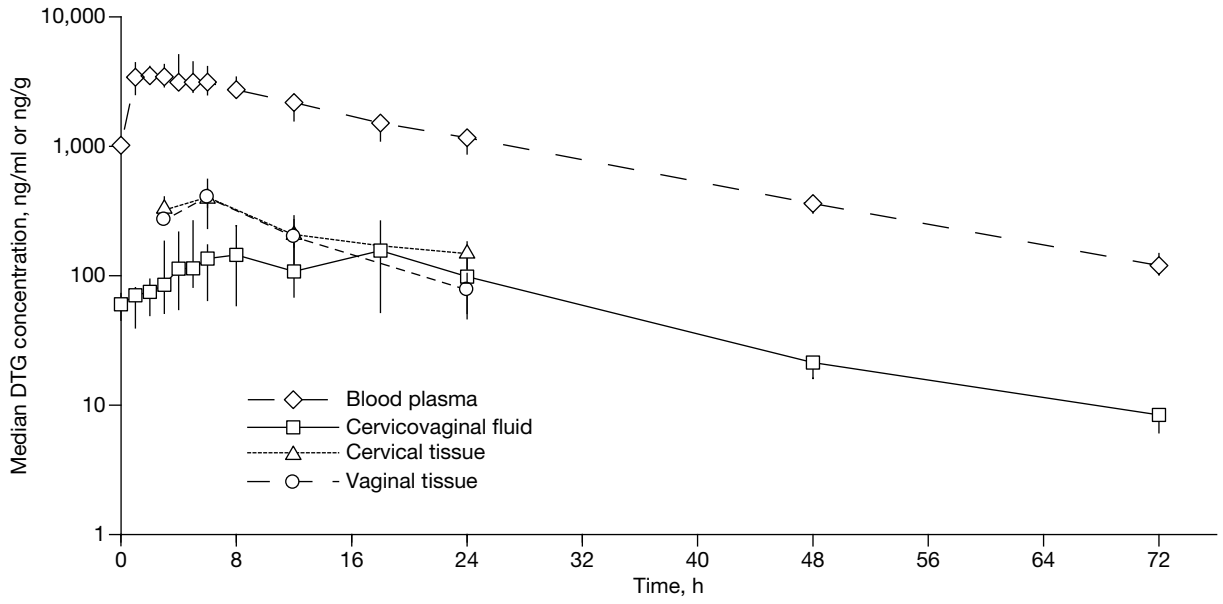
Cervicovaginal fluid concentrations

After a single dose, DTG was detected in CVF at 1 h post-dose in 3/8 women, and at 3 h post-dose in 8/8 women. The median (IQR) $AUC_{0–24\,h}$ for PK1 was 2,600 ng•h/ml (1,280–8,100) and for PK2 was 3,160 ng•h/ml (2,820–5,220). Over 24 h following the first dose, an elimination phase was not evident in 4/8 women, and a reliable $t_{½}$ could not be calculated. After 5–7 days of dosing, the median (IQR) $t_{½}$ was 13.5 h (11.1–14.9). The median (IQR) accumulation ratio of DTG in CVF was 0.82 (0.71–1.12) indicating no significant accumulation in the CVF with multiple dosing. After a single dose, the $AUC_{0–24\,h}$ penetration ratios of CVF:BP were 0.07 (0.05–0.18); after multiple dosing, the ratios were 0.06 (0.04–0.11). These data demonstrate that CVF exposure is approximately 6–7% of BP exposure.

Genital tract tissue concentrations

DTG concentrations were similar between CT and VT, after both single and multiple dosing (Spearman's

Figure 2. Pharmacokinetics of dolutegravir in blood plasma, cervicovaginal fluid, cervical tissue and vaginal tissue at steady state



At steady state, the median (IQR) dolutegravir (DTG) concentrations at each time point are plotted for blood plasma (BP) and cervicovaginal fluid (CVF) and the median (range) DTG concentrations of the two individual subject cervical tissue (CT) and vaginal tissue (VT) samples at each time point are plotted as a composite pharmacokinetic curve. Error bars represent IQR for BP and CVF, and the two individual samples at each time point for CT and VT. The area under the concentration–time curve (AUC) ratio of CVF to BP is 0.06 (IQR 0.04–0.11) at steady state and the tissue concentrations are 46–63% higher than in CVF.

ρ=0.70, *P*=0.003; Figure 3). For CT, the composite $AUC_{0-24\ h}$ for PK1 and PK2 was 2,750 ng•h/ml and 5,300 ng•h/ml, respectively. The accumulation ratio was 1.93. For VT, the composite $AUC_{0-24\ h}$ for PK1 and PK2 was 2,730 ng•h/ml and 4,740 ng•h/ml, respectively. The accumulation ratio was 1.74. These data suggest greater drug accumulation of DTG in female genital tract tissues than in BP. Penetration ratios for VT and CT, in relation to BP, were both 0.07 after a single dose, and were 0.09–0.10 after multiple dosing. After a single dose of DTG, VT and CT exposure was nearly double CVF concentrations at the same time points (CT:CVF ratio =1.97; VT:CVF ratio =1.95). After multiple dosing, tissue concentrations were 2–3× higher than CVF (CT:CVF ratio =2.61; VT:CVF ratio =2.34). The correlation between CVF and GT was 0.15 (*P*=0.55) at PK1, but the correlation between CVF and GT was 0.52 (*P*=0.04) at PK2 (Figure 4).

Adverse events

Adverse event data include all 11 enrolled women who received ≥1 dose of DTG. There were no serious adverse events and no women discontinued DTG as the result of an adverse effect. Non-serious adverse events that were grade 1 or 2 and determined to be possibly related to study drug involved 9 events in 8 women, and included headache (2 subjects), drowsiness (1 subject), dizziness/nausea (2 subjects), GI upset with gas (1 subject), lightheadedness upon standing (1 subject) and a grade 2 aspartate aminotransferase elevation at follow-up (1 subject). One subject experienced scalp tingling and it was unknown whether this was related to DTG. Adverse events that were determined to be unrelated to DTG administration included one episode of fainting in one subject with IV insertion prior to the first dose of DTG, irritation at the IV site (1 subject) and early menses (1 subject).

Discussion

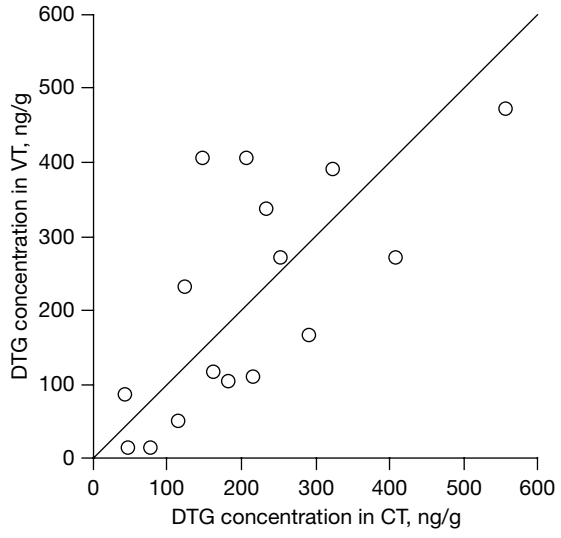
The recently published trial, HPTN052, provided proof that effective antiretroviral treatment of HIV-infected individuals decreases the risk of HIV transmission to negative partners by 96% [8]. Evidence from three large clinical trials also demonstrates that TDF and FTC taken orally by HIV-negative individuals can protect against infection through heterosexual or homosexual transmission [9–11]. Evidence also exists, however, that not all antiretrovirals penetrate the female genital tract equally well [12] and that viral shedding in the female genital tract can persist in patients who are have undetectable HIV RNA in BP while on antiretroviral therapy [6,7]. Of importance to both treatment as prevention and pre-exposure

Table 1. Pharmacokinetic parameters of dolutegravir in blood plasma, cervicovaginal fluid, cervical tissue and vaginal tissue

Parameter	PK1, median (IQR)	PK2, median (IQR)	Accumulation ratio[a]
Blood plasma			
$AUC_{0-24\ h}$, ng•h/ml	38,300 (31,600–45,400)	55,400 (43,300–62,200)	1.42 (1.28–1.50)
C_{last}, ng/ml	821 (610–956)	120 (102–149)	–
C_{max}, ng/ml	3,000 (2,380–3,470)	3,770 (3,430–5,250)	–
T_{max}, h	2.0 (1.0–4.8)	2.5 (1.3–4.0)	–
$T_{½}$, h	Not calculated[b]	14.8 (13.6–16.1)	–
Cervicovaginal fluid			
$AUC_{0-24\ h}$, ng•h/ml	2,600 (1,280–8,100)	3,160 (2,820–5,220)	0.82 (0.71–1.12)
C_{last}, ng/ml	93.1 (47.2–122)	8.7 (6.6–10.2)	–
C_{max}, ng/ml	149 (84.4–435)	230 (93.4–347)	–
T_{max}, h	9.9 (5.7–17.9)	7.9 (3.7–11.9)	–
$t_{½}$, h	Not calculated[b]	13.5 (11.1–14.9)	–
Cervical tissue			
$AUC_{0-24\ h}$, ng•h/g	2,750	5,300	1.93
C_{last}, ng/g	59.6 (41.6–77.5)	149 (114–183)	–
C_{max}, ng/g	236 (148–324)	395 (232–557)	–
T_{max}, h	6	6	–
$t_{½}$, h	Not calculated[b]	Not calculated[b]	–
Vaginal tissue			
$AUC_{0-24\ h}$, ng•h/g	2,730	4,740	1.74
C_{last}, ng/g	52.1 (16.0–88.1)	77.8 (51.1–105)	–
C_{max}, ng/g	399 (392–407)	405 (337–472)	–
T_{max}, h	6	6	–
$t_{½}$, h	Not calculated[b]	Not calculated[b]	–

Tissue areas under the concentration–time curves (AUCs) are composite geometric means of all subjects. [a]Data for blood plasma and cervicovaginal fluid are median (IQR) of the ratio of AUC at steady state (PK2)/AUC at first dose (PK1) and for cervical tissue and vaginal tissue are composite AUC over 24 h ($AUC_{0-24\ h}$) at PK2/composite $AUC_{0-24\ h}$ at PK1. [b]Half-life ($t_{½}$) was not calculated for blood plasma or cervicovaginal fluid after composite PK1 because there was not a clear elimination phase in all subjects by 24 h and was not calculated for cervical or vaginal tissue at either time point because each individual subject only had one sampling time and concentrations remained high at 24 h. C_{last}, concentration at 24 h for PK1 and concentration at 72 h for PK2; C_{max}, maximum concentration; T_{max}, time to maximum concentration.

Figure 3. The correlation between cervical tissue and vaginal tissue dolutegravir concentrations



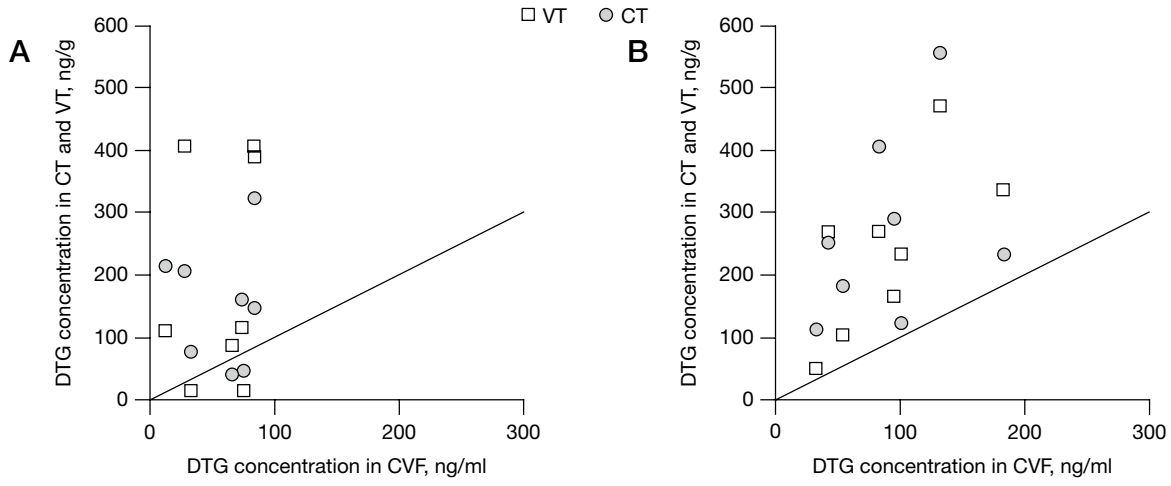
Individual concentration are plotted for dolutegravir (DTG) in cervical tissue (CT) and vaginal tissue (VT). A significant (P=0.003) correlation is noted between CT and VT concentrations (ρ=0.70). A line of unity is presented as a reference.

prophylaxis strategies, are antiretroviral agents with long half-lives that allow for once-daily dosing, with favourable adverse effect profiles and with the ability to penetrate the mucosal compartments involved in HIV transmission (for example, the female genital tract).

In this study, BP exposures of DTG after single and multiple dosing were found to be consistent with previously published data [13]. Concentrations above the established protein binding-adjusted 90% inhibitory concentration (IC_{90}) of 64 ng/ml were achieved within 1 h of the first dose and remained well above 64 ng/ml for the entire dosing interval within all subjects [14]. In 7/8 subjects, BP concentrations remained above the IC_{90} at 72 h after the final dose. These data demonstrate prolonged exposure post-dose, and some degree of adherence 'forgiveness'. No serious adverse events were seen in this study. The adverse effects that were reported were mild, and did not lead to discontinuation of DTG.

CVF concentrations were detectable 3 h after dosing in all subjects, and remained detectable through 72 h after the last dose. Overall DTG exposure was approximately 6% of BP, with a CV% of 72% at steady state. In

Figure 4. The correlation between cervicovaginal fluid and genital tissue dolutegravir concentrations



Individual concentration/time points are plotted for dolutegravir (DTG) in genital tract tissues and in cervicovaginal fluid (CVF). **(A)** Cervical tissue and vaginal tissue correlated with CVF following the first dose of DTG. **(B)** Cervical tissue and vaginal tissues correlated with CVF after multiple dosing. The correlation between CVF and genital tissues (GT) at PK1 is 0.15 (P=0.55), and between CVF and GT at PK2 is 0.52 (P=0.04). A line of unity is presented as a reference.

BP, DTG is >99% protein bound. Lopinavir, atazanavir, amprenavir, ritonavir and EFV, which are also highly protein bound (>85%), also have low penetration into CVF compared to BP [12,15]. Raltegravir, which has 83% protein binding and exhibits more variable BP PKs (coefficient of variation =212%) [16], has CVF exposures that are 100–400% of BP [17–19]. Elvitegravir, the other INSTI currently on the market, has yet to establish female genital tract exposure. However, it is also highly protein bound in BP (98%), and requires a PK booster in order to achieve a $t_{½}$ adequate for once-daily dosing [20].

Protein binding in the CVF was not specifically measured in this study. If we assume equivalent protein binding in CVF as in BP for DTG (>99%), concentrations above the established protein binding-adjusted IC_{90} of 64 ng/ml were achieved within 6 h of the first dose for 50% of the subjects and remained >64 ng/ml for the entire dosing interval in those same subjects. However, the major drug-binding proteins α-1-acid glycoprotein and albumin, have concentrations in CVF that are <1% of what is measured in BP [21]. Additionally, we have measured maraviroc CVF protein binding, and have found it to be 10% of what is seen in BP (7.6% versus approximately 76%) [22,23]. Since the protein-unbound DTG 50% inhibitory concentration (IC_{50}) is 0.21 ng/ml, if ≥90% of DTG in CVF is unbound, it is likely that CVF exposures are above this value in all women by 4 h after a single dose, and maintained in all women after multiple dosing out to at least 72 h post-dose.

DTG concentrations in CVF correlated with CT and VT at steady state, suggesting CVF may be a useful surrogate for tissue exposures. However, tissue sampling occurred at only a small number of time points (1 per subject), and should be investigated further to establish the relationship. DTG concentrations were similar in VT and CT, suggesting equal distribution of drug throughout the lower female genital tract. Penetration ratios for VT and CT, in relation to BP, were 0.07 after a single dose and 0.09–0.10 after multiple dosing. Female genital tract tissue:plasma AUC ratios for other antiretrovirals range from 0.6 (TDF) to 42 (FTC) [7]. DTG concentrations in these tissues were >IC_{90} in all eight women at all four steady-state time points.

Additionally, a Phase II dose ranging study of DTG identified a maximum effect (E_{max}) model that best explained the relationship between DTG trough concentrations and efficacy. The E_{max} IC_{50} was 36 ng/ml. Trough concentrations were above this value in plasma in all subjects at 24 and 72 h post-dose. In CVF, trough concentrations >36 ng/ml were achieved in 7/8 subjects at 24 h, but in no subjects by 48 h. All CT and VT concentrations were >36 ng/ml after multiple dosing [13].

While it is important to investigate the PKs in the female genital tract of healthy women, it would also be of interest to determine the PKs and viral load suppression achieved in HIV-infected women to determine the potential role of DTG in treatment as prevention. Given the similar plasma concentrations of DTG in both healthy subjects and the HIV-infected population,

a significant difference in genital tract penetration of DTG would not be expected [13,14].

In conclusion, DTG plasma PK parameters after single and repeat doses were similar to previously reported data. DTG exposure in CVF was 6% of plasma exposure under steady-state conditions. Delayed T_{max} was observed in CVF (T_{max}=6 h) compared to plasma (T_{max}=2 h). There was only marginal accumulation after repeat dosing. DTG exposure in CT and VT were similar and appoximately 10% of plasma exposure under steady-state conditions. Higher accumulation of DTG after multiple dosing was noted in both CT and VT compared to BP. DTG concentrations were above the protein binding-adjusted IC_{90} in 100% of CT and 88% of VT samples. DTG was well tolerated with no significant safety issues observed.

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JLA contributed to subject recruitment and study visit conduct, PK and statistical data analysis, and was the primary author of the manuscript. KBP contributed to study visit conduct, was the clinical study safety officer, and contributed to critical review of manuscript. HMAP contributed to subject recruitment and study visit conduct, and critical review of manuscript. CS contributed to analytical data analysis and critical review of manuscript. BNG contributed to study visit conduct, PK and statistical data analysis, and critical review of manuscript. JBD contributed to PK and statistical data analysis, and critical review of manuscript. ADMK contributed to study design, analytical and PK data analysis, funding support and critical review of the manuscript.

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