Genital HIV-1 Shedding With Dolutegravir (DTG) Plus Lamivudine (3TC) Dual Therapy

To the Editors:

INTRODUCTION

Detection of HIV RNA in the genital tract is correlated with sexual transmission[1–4] and is best predicted by the degree of plasma viremia.[5] Although there is a near-linear relationship between blood and genital HIV RNA, episodic genital HIV RNA expression occurs in some individuals with suppressed viremia possibly due to genital viral compartmentalization with poor drug penetration,[6,7] or stimulation of virus replication by sexually transmitted infections and genital inflammation.[8–10] Furthermore, discordant viral resistance patterns in blood and genital samples occur in some individuals,[11] supporting the possibility of differential virus evolution in these anatomical compartments. Although genital HIV RNA shedding has been reported in 2%–20% of individuals on standard 3-drug antiretroviral therapy (ART),[8,9,12,13] there is no evidence that such shedding leads to new infections in the context of suppressed viremia, hence the recent consensus statement, "undetectable equals untransmittable" or "U = U."[14]

Several 2-drug regimens are being studied because of potential benefits of reduced antiretroviral exposure and costs. Enthusiasm for 2-drug regimens may be limited if they lead to increased genital HIV shedding. Thus, we investigated genital HIV RNA shedding with dolutegravir (DTG) plus lamivudine (3TC), which is under investigation for initial and maintenance therapy.

METHODS

Study Participant Characteristics and Clinical Sampling

We recruited participants from 2 clinical trials: (1) virologically suppressed participants (for >48 weeks at the time of screening) randomized to continuation of 3-drug ART or switch to DTG+3TC maintenance in Antiretroviral Study to Promote Improvement and Reduce Exposure (ASPIRE),[15] and (2) ART-naive participants, who initiated DTG+3TC in the single-arm AIDS Clinical Trials Group (ACTG) A5353 phase 2 pilot study.[16]

At study weeks 24 (or 36) and 48, female genital secretions were collected using self-administered vaginal swabs and immediately frozen at −80°C, whereas male genital secretions were collected by masturbation. Seminal plasma was processed according to standard procedures and stored at −80°C.[5] The parent studies (ie, ASPIRE and A5353) and this genital substudy were approved by the Institutional Review Board at each study site, and each participant provided a written informed consent.

RNA Extraction From Genital Secretion and HIV Quantification

HIV RNA levels were measured in seminal plasma, as previously described.[17,18] For female secretions, RNA was recovered from dry vaginal swabs by suspending with 1 mL of RPMI 1640 Medium vortexed and incubated for 10 minutes on ice. Supernatant was centrifuged (23,500*g* at 4°C for 1 hour). Concentrated RNA was extracted using High Pure Viral RNA Kit (Roche), and cDNA was generated using the SuperScript III First-Strand Synthesis Kit (Invitrogen).[17,19] HIV RNA was quantified by real-time PCR in an ABI 7900HT thermocycler (Applied Biosystems).[17,19]

DNA Extraction From Genital Secretion and Herpesvirus DNA Quantification

Viral DNA was extracted from 200 μL of seminal plasma and 400 μL dry swab resuspension with 1X PBS using QIAamp DNA Mini Kit (Qiagen) per manufacturer's protocol. Four hundred microliter DNA from dry swab was further concentrated by standard ethanol-based DNA precipitation. EDTA (50 mM) was quickly added to raw semen samples to inhibit DNase activity.[18] Levels of different herpesviruses in semen were measured by real-time PCR in an ABI 7900HT thermocycler (Applied Biosystems).[17,19]

Genotyping

Extracted RNA was reverse transcribed using the SuperScript III Reverse Transcriptase (Thermo Fisher Scientific). Nested PCR was performed to obtain integrase or protease/reverse transcriptase using Taq HiFi polymerase (invitrogen) and Sanger sequenced. Sequences were manually edited using BioEdit Sequence Editor (v7.0.5) and evaluated for the presence of drug resistance mutations using the HIVseq Program from the Stanford University Drug Resistance Database.

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TABLE 1. Summary of HIV RNA Shedders (N = 3)

Parent Study	Study, wk	ART, Regimen	Last Missed Doses During Study	Genital HIV RNA (Copies/mL)	Plasma HIV RNA (Copies/mL)*	CMV DNA (Copies/mL)	HSV DNA (Copies/mL)	Gonorrhea RNA	Chlamydia RNA
ASPIRE #1	48	RPV/ TDF/ FTC	1–2 wk	42	179	Not detected	Not detected	Not detected	Not detected
ASPIRE #2†	36	DTG +3TC	>3 mo	488	<20	314,607	Not detected	Not detected	Not detected
	48	DTG +3TC	Never	79	31	86,090	Not detected	Not detected	Not detected
A5353	24	DTG +3TC	Never	48	<40	NA‡	NA‡	Not detected	Not detected

*Plasma HIV RNA collected at the same time as genital HIV RNA shedding.
†ASPIRE participant #2 had detectable HIV RNA at 2 consecutive time-points (weeks 36 and 48).
‡Genital HSV and CMV testing could not be completed in one participant with HIV genital shedding because there was not enough seminal plasma to perform this analysis.
3TC, lamivudine; DTG, dolutegravir; FTC, emtricitabine; NA, not available; RPV, rilpivirine; TDF, tenofovir.

Statistical Analysis

Genital HIV RNA above the lower limit of detection (40 copies/mL) was considered detectable. The proportion of participants with detectable HIV RNA was determined in each study arm, and 95% confidence intervals were calculated using binomial exact test. No formal statistical comparisons were performed because of the limited sample size.

RESULTS

Study Participant Characteristics

We enrolled 38 participants from ASPIRE (18 switched to DTG+3TC while 20 had remained on their 3-drug regimen), and 13 ART-naïve individuals who initiated DTG+3TC in the ACTG A5353 study. The median ART duration before ASPIRE entry was 5.4 years (IQR: 3.5–7.6) in the DTG+3TC group and 6.2 years (3.8–7.8) in the 3-drug group. The prerandomization regimens included a protease inhibitor (32%), non-nucleoside reverse transcriptase (26%) or integrase inhibitor (42%). Participants' characteristics are summarized in Table 1, Supplemental Digital Content, http://links.lww.com/QAI/B229.

HIV RNA Shedding

A total of 76 seminal fluid samples were collected from 45 men (20 at week 24, 15 at week 36, and 41 at week 48). Twelve vaginal swabs were collected from 6 women (4 at week 24, 2 at week 36, and 6 at week 48). All but 3 participants had undetectable HIV RNA in blood plasma at each time-point. Three male participants, and no female participants, had HIV RNA genital shedding >40 copies/mL (Table 1). They included 1/20 participants (5%, 95% CI) in the ASPIRE 3-drug arm with genital HIV RNA of 42 copies/mL at week 24, 1/18 participants [5.6%, 95% CI: (0.1% to 27%)] in the ASPIRE DTG+3TC arm with detectable genital HIV RNA at 2 consecutive time-points (488 copies/mL at week 36 and 79 copies/mL at week 48), and 1/13 participants [7.7%, 95% CI: (0.2% to 36%)] in ACTG A5353 with genital HIV RNA of 48 copies/mL at week 24, Table 1, Supplemental Digital Content, http://links.lww.com/QAI/B229 and Figure 1, Supplemental Digital Content, http://links.lww.com/QAI/B229. Two of 3 men with genital HIV shedding also had coincident viremia in plasma.

Gonorrhea and chlamydia RNA were not detected from any of the 3 HIV genital shedders. One HIV genital shedder (from the ASPIRE DTG+3TC group) had high levels of genital CMV DNA 314,607 copies/mL and 86,090 copies/mL at the 2 time-points where genital HIV RNA was detected. Genotyping of genital HIV isolates was unsuccessful because of the low sample volume and viral load, except integrase sequencing in 1 ASPIRE DTG+3TC participant (with 488 copies/mL HIV RNA in seminal fluid) that revealed no resistance mutations.

DISCUSSION

There is robust evidence from studies of 3-drug regimens that U = U ("undetectable equals untransmittable").[14] Less is known about the incidence of genital HIV RNA shedding and transmission prevention attributes of investigational 2-drug regimens. DTG+3TC is of particular interest because it could become a recommended option if its promising preliminary efficacy and safety results are confirmed in ongoing phase 3 trials.[20]

In our pilot study of 51 adults living with HIV, the frequency of genital HIV RNA shedding while virologically suppressed in blood was similar between those who were on standard 3-drug ART and those who were on DTG+3TC as initial or maintenance therapy. The frequency of genital shedding in each of these groups fell within the lower end of the range reported for 3-drug regimens in other studies (2%–20%).[8,9,12,13] None of the 6 women enrolled in our study had genital shedding. As reported by previous investigators,[5] we observed a concordance between viremia and genital shedding as 2 of 3 participants with detectable seminal HIV RNA also had viremia. Taken together, these results suggest that DTG + 3TC is effective in controlling genital HIV shedding, which accounts for most HIV transmission.[21]

In a previous study, the triple regimen of DTG+3TC+abacavir led to more rapid attainment of HIV RNA <40

copies/mL in semen than blood in treatment-naive individuals,[22] likely because of lower baseline HIV RNA levels in semen. It is unknown whether a similar relationship between blood and seminal virus decay exists with DTG +3TC dual therapy, and this could be formally assessed as part of a clinical trial. Nevertheless, good efficacy of DTG + 3TC in the genital compartment is pharmacologically plausible because seminal protein-unbound DTG concentrations exceed the in vitro 50% inhibitory concentration by a median of 214-fold despite lower concentrations in seminal fluid than blood,[22] whereas seminal fluid concentration of 3TC exceeds the plasma concentration.[7]

Our results are limited by the small sample size, the small number of women enrolled, and by the sparse timing of specimen collection. We also did not assess pre-treatment and post-treatment HIV RNA in each participant; hence, we are unable to describe the magnitude of genital viral load reduction with DTG + 3TC.

In conclusion, in this small pilot study, we did not detect concerning signals about the efficacy of the 2-drug regimen of DTG+3TC in controlling genital HIV RNA shedding, hence prevention of viral transmission, when HIV RNA is undetectable in blood plasma. These preliminary results suggest that DTG+3TC likely confers similar transmission prevention benefits as triple therapy.

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