

Short communication

Genital secretion HIV RNA shedding in HIV-positive patients on ritonavir-boosted protease inhibitor monotherapy or standard combination ART: a cross-sectional sub-study from the PIVOT Trial

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Background: Protease inhibitors (PI) have relatively low penetration into the genital tract, raising concerns about the potential for genital HIV RNA shedding in patients taking PI-based regimens, particularly PI monotherapy (PI-mono).

Methods: We measured HIV RNA and PI drug concentrations in samples of semen, cervico-vaginal and rectal mucosa secretions, and plasma in patients after 48–96 weeks on PI-mono or standard triple therapy.

Results: A total of 85 participants were recruited. Of the 43 participants on PI-mono (70% on darunavir [DRV]/ritonavir [r]), 3 had detectable virus in semen or vaginal secretions (all below quantification limit), and none in rectal mucosa or plasma. Among those taking triple therapy, five had detectable virus in semen or vaginal secretions (HIV RNA >50 copies/ml in one), none in rectal mucosa and one in plasma.

The median (IQR) concentration of DRV and atazanavir in semen (659.7 [339–1,089] and 128.8 [63–368] ng/ml, respectively) and cervico-vaginal samples (2,768 [312–7,879] and 1,836 [359–3,314] ng/ml, respectively) exceeded their protein adjusted median inhibition concentration (MIC₅₀). DRV concentration in rectal secretions showed higher variability compared with concentration in the other sites, with particularly high rectal secretion/blood ratios (median 8.4, IQR 2.6–68.7:1).

Conclusions: We found no substantive evidence of HIV shedding in patients taking PI-mono, suggesting that PIs provide adequate control of virus in the genital compartment and are unlikely to lead to ongoing sexual transmission.

Introduction

Monotherapy with ritonavir boosted protease inhibitors (PI-mono) has been investigated as a treatment simplification strategy in effectively suppressed

HIV-positive patients and shown to be safe with regular viral load monitoring and prompt reintroduction of combination therapy when needed [1–3].

Although most patients on PI-mono maintain effective viral suppression in blood plasma, concerns have been expressed about the possibility of occult viral replication in the genital tract because of the relatively poorer penetration of some PIs into semen [4,5] or cervico-vaginal secretions [6,7] particularly in the absence of any other effective drug.

To investigate the frequency of HIV RNA shedding in genital secretion in patients randomized to ritonavir-boosted PI-mono compared with continuing on standard triple combination ART we conducted a cross-sectional study in a sub-set of patients taking part in a large clinical trial [1].

Methods

Sub-study population

Participants were taking part in the PIVOT trial ($n=587$) in which HIV-positive adults taking standard combination antiretroviral therapy (ART) with no history of virological failure were randomized to either continue on ongoing triple therapy (OTT) or to be switched to ritonavir-boosted PI-mono and followed-up for a median of 44 months [1,8]. Treatment was open label. Study participants from both randomization arms were invited to join this sub-study at either week 48 or 96 after randomization. At the sub-study visit, participants were asked about symptoms associated with sexually transmitted infections

Table 1. Study population

	PI-mono ($n=43$)	OTT ($n=42$)	Total ($n=85$)
Sex			
Male, n (%)	34 (79.1)	33 (78.6)	67 (78.8)
Female, n (%)	9 (20.9)	9 (21.4)	18 (21.2)
Median age in years at main study entry (IQR)	45 (41–49)	46 (39–50)	46 (40–49)
ART at the time of the sub-study visit			
2NRTI/1NNRTI, n (%)	5 (12)	25 (59)	30 (35)
2NRTI/1PI/r, n (%)	5 (12)	16 (39)	21 (25)
ATV/r, n (%)	2 (5)	0	2 (2)
DRV/r, n (%)	30 (69)	1 (2)	31 (37)
LPV/r, n (%)	1 (2)	0	1 (1)
Sexually transmitted infections			
Screened for, n (%)	24 (55.8)	26 (61.9)	50 (58.8)
Diagnosed with, n (%)	3 (7.0)	1 (2.4)	4 (4.7)
Genital secretions			
Semen, n	28	29	57
Rectal, n	34	27	61
Vaginal, n	9	9	18

ATV/r, ritonavir-boosted atazanavir; DRV/r, ritonavir-boosted darunavir; LPV/r, ritonavir-boosted lopinavir; NNRTI, non-nucleoside reverse transcriptase inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; OTT, ongoing triple therapy; PI-mono, protease inhibitor monotherapy.

(STI) and screened for these, if appropriate, according to standard clinical pathways at each site.

Sample collection, processing and analysis

Semen samples were self-collected and processed within 4 h of being produced. Cervico-vaginal secretion (CVS) and rectal mucosal secretion (RMS) samples were collected by research staff using Weck Cel® ophthalmic spears (Beaver-Visitec International, Inc., Waltham, MA, USA), placed in pre-filled vials with RNAlater (Ambion, Inc., Foster City, CA, USA) and phosphate-buffered saline (PBS; Sigma-Aldrich, Gillingham, UK), and stored at -80°C for batch testing at the end of the main study period. A plasma sample was also drawn at the same sub-study visit for immediate HIV RNA testing and storage.

HIV RNA was measured in semen, CVS and RMS samples at the University College London Hospital virology laboratory, using an in-house developed real-time PCR method that had previously been validated for the type of samples tested in this sub-study. Plasma HIV RNA was measured using standard tests at each clinical site. All samples with detectable HIV RNA, even if below the accurate level of quantification for the method, were tested for genotypic drug resistance.

Concentration of PIs in semen, CVS, RMS and in stored plasma samples was measured at the Bioanalytical Facility, University of Liverpool using high performance liquid chromatography–mass spectrometry [9]. Drug levels were expressed as median and ranges, as well as a genital/rectal:plasma ratio.

The sub-study was approved by the Cambridgeshire 4 Research Ethics committee.

Results

85 participants were recruited (43 on PI-mono; 67 male; median age 46 years). Demographic characteristics were similar between treatment groups (Table 1). In the 42 on triple therapy, 25 (60%) were taking non-nucleoside reverse transcriptase inhibitor (NNRTI)-based combination ART. In addition to CVS, 4 of the 18 female participants (22%) also provided RMS (Table 1). No participants reported urethral or rectal discharge; two reported vaginal discharge (both on PI-mono). Of the 50 screened for STIs, two had genital warts (PI-mono), two genital ulcers of unknown aetiology (triple therapy), one urethral gonorrhoea (PI-mono), one acute syphilis (PI-mono) and two HSV infection (one PI-mono, one triple therapy).

Virology data

Of the 57 semen samples obtained, HIV RNA was detectable in 5 (8.8%; 2 PI-mono, 3 triple therapy)

but below the limit for quantification. HIV RNA was <50 copies/ml in all paired plasma. Of the 18 CVS samples obtained, HIV RNA was detectable in 3 (16.7%; 2 triple therapy) but only 1 of these (triple therapy) was above quantification level (118 copies/ml). HIV RNA in the paired plasma sample from that participant was 56 copies/ml but was <50 copies/ml in the other two participants. The participant with detectable HIV RNA in CVS was receiving abacavir/lamivudine/efavirenz and had no symptoms suggestive of any STI. Without any ART change her HIV RNA in plasma was <50 copies/ml at the following study visit.

All 61 RMS samples had undetectable HIV RNA levels and all but one of the paired plasma samples from these participants also had HIV RNA <50 copies/ml. The only participant with detectable plasma HIV RNA in this group was the same female participant with detectable HIV RNA in CVS. Of the eight participants with detectable HIV RNA in semen or CVS samples, only one (CVS) had an STI screen performed at the sub-study visit, which was negative.

Genotypic resistance testing was attempted in eight samples (five semen and three CVS) with detectable HIV RNA and sequencing was successful in four. The sequence from all semen samples was wild type; the sequence from one CVS sample showed E138A (on triple therapy; the same mutation was present in a plasma sample drawn 5 years earlier).

Pharmacokinetic data

PI concentration was measured in plasma samples from 54 participants on darunavir (DRV) or atazanavir (ATV). Of these, 12 female participants provided CVS samples, 9 were on DRV (all PI-mono) and 3 on ATV (all OTT). Of the 41 semen samples tested, 23 corresponded to participants on DRV (21/28 PIM) and 5 on ATV (all OTT). The concentration of darunavir (DRV) and atazanavir (ATV) in semen (median 659.7 ng/ml and 128.8 ng/ml, respectively) and CVS (median 2,768 ng/ml and 1,836 ng/ml, respectively) exceeded the DRV and ATV protein adjusted MIC₅₀ (Figure 1). PI concentration in RMS showed higher variability compared with concentration in the other sites, with DRV especially showing high rectal secretion/blood ratios (median 8.4; IQR 2.6–68.7:1).

Out of the three CVS with detectable HIV RNA, only one was obtained from a participant on PI-mono (DRV/r). At week 48, this participant had HIV RNA detectable below the quantification level of the assay but had DRV levels well above the MIC₅₀ in CVS in the study (38,214 ng/ml). Of the five participants with detectable HIV RNA in semen (below the level accurate quantification of the assay), only two were on PI-based ART, one on DRV/r monotherapy (drug levels

not measured) and the other on ATV/r-based combination ART (drug levels in excess of MIC₅₀). The median time between last ART dose and sub-study samples collection was 12.2 h (IQR 3.8–14.9) in participants taking DRV and 15.7 h (IQR 7.0–23.5) in those receiving ATV.

Discussion

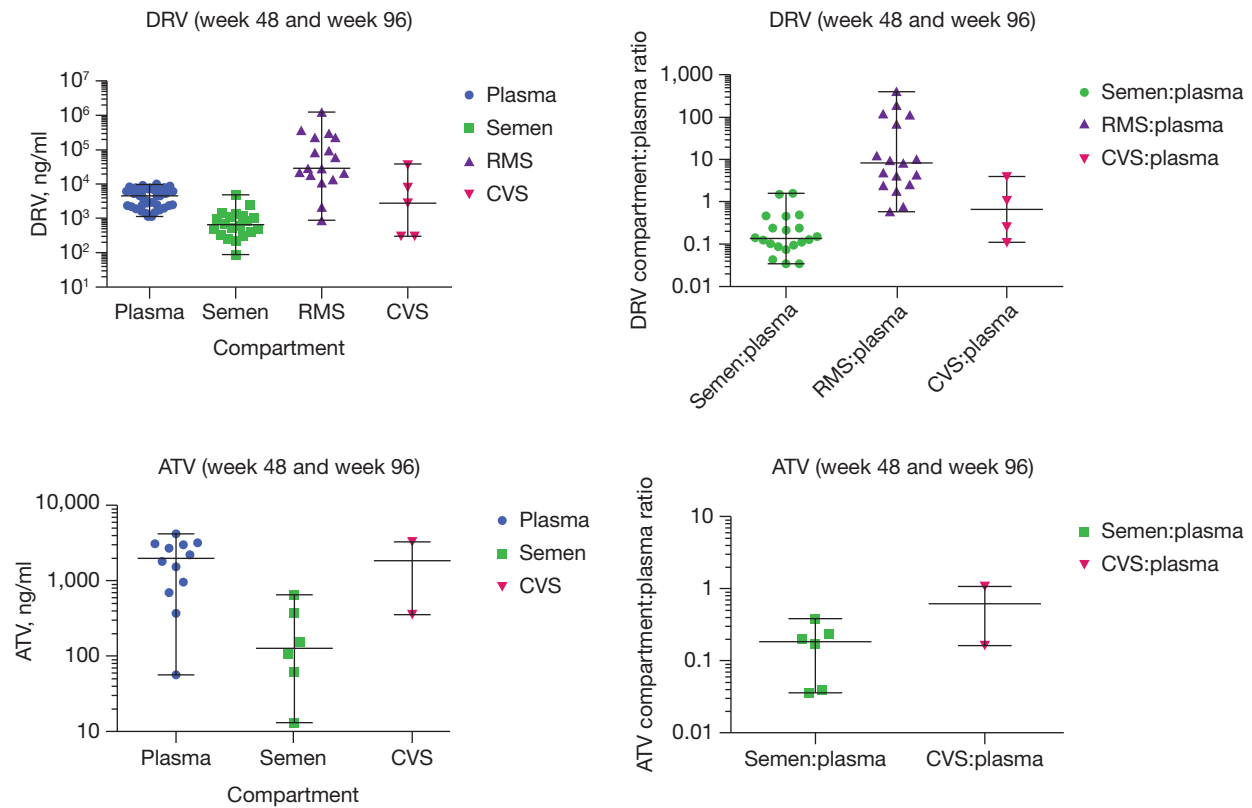
We found no evidence of substantive genital secretion HIV RNA shedding in virologically suppressed HIV-positive patients on PI-mono, which is consistent with previous reports [10,11]. Indeed, the overall prevalence of detectable HIV RNA (below the level of accurate quantification of the assay) was low in genital secretion samples, regardless of treatment arm and/or presence of concurrent STI, a known risk factor for HIV RNA rebound in the genital tract [12,13].

The participant with HIV RNA above 50 copies/ml in both the CVS and plasma had very low viral load in both compartments (that is, <200 copies/ml), but it was somewhat higher in the genital tract than in plasma, which is also consistent, although at much lower level than previously reported [14]. Our results for DRV/ATV (corrected to ng/ml of volume) in CVS samples were consistent with the data from direct cervico-vaginal aspirates [6].

Antiretroviral drug penetration in the genital tract seems to be drug rather than class dependent, but overall, PIs tend to reach lower concentrations than other antiretroviral drugs in the genital tract [15]. However, among PIs, DRV exhibits the highest penetration rate to female genital tract [16]. In our study the concentration of DRV and ATV in genital/rectal samples were well above the MIC₅₀ in all cases but we observed very high concentrations of DRV in rectal mucosa samples. This could either be due to high penetration of drug via the rectal mucosa or unabsorbed drug in the rectal lumen, as over 40% of DRV is eliminated unchanged in faeces [17]. Concentration of DRV in rectal tissue has been reported to be 2.3- to 2.7-fold higher than in plasma in HIV-negative men [18].

Although done as part of a rigorously conducted, randomized controlled trial, the subgroup studied is small (less than 15% of trial participants) and the invasive nature of the sub-study may have resulted in some selection bias. However, the study was conducted at 12 large treatment centres across England and no obvious differences were found between those who participated in the sub-study and those who did not. STI screening was not conducted in all participants but only in those symptomatic and that may partially explain the relatively low STI prevalence we identified compared with other similar populations [19] and this may also limit the generalizability of the findings. However, recent data suggest that rectal bacterial STIs are

Figure 1. Protease inhibitor concentration (protease inhibitor monotherapy and triple therapy groups combined)



ATV, atazanavir; CVS, cervico-vaginal secretion; DRV, darunavir; RMS, rectal mucosal secretion.

not associated with HIV shedding in patients effectively suppressed on ART [20]. Furthermore, in the PIVOT trial, the initial rate of viral rebound on PI-mono was high (32%). This peaked at month 9, declining subsequently and stabilizing at a much lower level by month 18 [21]. It is therefore possible that patients at highest risk of genital compartment rebound on PI-mono are likely to have been re-suppressed by reinduction with triple therapy by the time of the sub-study sampling. Sampling at an earlier time point may have revealed a higher proportion with detectable viral replication. However, shedding of virus in genital compartment may be episodic and relatively brief, so frequent repeat longitudinal sampling would be needed to detect this.

Despite the cross-sectional nature of this sub-study and its relatively small sample size, its results show no excess genital secretion HIV RNA shedding in virologically suppressed patients treated with ritonavir-boosted PI monotherapy as compared with combination ART, suggesting that PI-mono is unlikely to lead to ongoing

sexual transmission. This may also apply to PI-based dual therapy combinations.

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Disclosure statement

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Additional file

Additional file 1: Supplemental acknowledgements can be found at https://www.intmedpress.com/uploads/documents/4534_Arenas_Pinto_Addfile1.pdf

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