INTERNATIONAL MEDICAL PRESS

Antiviral Therapy

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

Alejandro Arenas-Pinto, Wolfgang Stöhr, Saye Khoo, Amanda Clarke, Nicholas Beeching, Zoe Warwick, Vincent Lee, Laura Else, Rebecca Wiggins, Bridget Ferns, Eleni Nastouli, David Dunn, Charles J Lacey, Nicholas I Paton, the Protease Inhibitor monotherapy Versus Ongoing Triple therapy (PIVOT) Genital Secretion sub-study Team

Antiviral Therapy 2020; 10.3851/IMP3340

Submission date 26th April 2019
Acceptance date 17th October 2019
Publication date 23rd March 2020

This provisional PDF matches the article and figures as they appeared upon acceptance. Copyedited and fully formatted PDF and full text (HTML) versions will be made available soon.

For information about publishing your article in *Antiviral Therapy* go to http://www.intmedpress.com/index.cfm?pid=12

©2020 International Medical Press

ISSN 1359-6535

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP3340

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

Alejandro Arenas-Pinto[1,2], Wolfgang Stöhr[1], Saye Khoo[3,4], Amanda Clarke[5,6], Nicholas Beeching[4], Zoe Warwick[7], Vincent Lee[8], Laura Else[3], Rebecca Wiggins[9], Bridget Ferns[10], Eleni Nastouli[10], David Dunn[1,2], Charles J Lacey[9], Nicholas I Paton[1,11], the Protease Inhibitor monotherapy Versus Ongoing Triple therapy (PIVOT) Genital Secretion sub-study Team[†]*

[1]MRC-Clinical Trials Unit at UCL, London, UK

[2]UCL Institute for Global Health, London, UK

[3]Institute of Translational Medicine, University of Liverpool, Liverpool, UK

[4]Royal Liverpool University Hospital, Liverpool, UK

[5]Brighton & Sussex University Hospitals NHS Trust, Brighton, UK

[6]Sussex Medical School, Brighton, UK

[7]Derrifor Hospital, Plymouth, UK

[8]Manchester Royal Infirmary, Manchester, UK

[9]York Biomedical Research Institute, University of York, York, UK

[10]Department of Virology, University College London Hospitals NHS Foundation Trust, London, UK

[11]Yong Loo Lin School of Medicine, National University of Singapore, Singapore

*Corresponding author e-mail: a.arenas-pinto@ucl.ac.uk

[†]The members of the PIVOT Genital Secretion sub-study Team are listed in the Acknowledgements section

Abstract

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 (PImono).

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 PImono or standard triple therapy.

Results: A total of 85 participants were recruited. Of the 43 participants on PImono (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, 5 had detectable virus in semen

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP3340

or vaginal secretions (HIV-RNA >50 copies/ml in one), none in rectal mucosa, and one in plasma.

The median (IQR) concentration of darunavir and atazanavir in semen (659.7 (339 – 1089) and 128.8 (63 – 368) ng/mL respectively) and cervico-vaginal samples (2768 (312 – 7879) and 1836 (359 – 3314) ng/mL respectively) exceeded their protein adjusted median inhibition concentration (MIC_{50}). Darunavir (DRV) concentration in rectal secretions showed higher variability compared to 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 monotherapy, suggesting that protease inhibitors provide adequate control of virus in the genital compartment and are unlikely to lead to ongoing sexual transmission.

Accepted 17 October 2019, published online 23 March 2020.

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 randomised 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 anti-retroviral therapy (ART) with no history of virological failure were randomised to either to 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 randomisation arms were invited to join this sub-study at either week 48 or 96 after randomisation. At the sub-study visit, participants were asked about symptoms associated with sexually transmitted infections (STI) and screened for these if appropriate according to standard clinical pathways at each site.

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP3340

Samples collection, processing and analysis

Semen samples were self-collected and processed within four hours of being produced. Cervico-vaginal (CCVS) 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 prefilled 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 (Supplemental table 1). 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, cervico-vaginal and rectal secretion 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 (Supplemental table 2). 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). 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). 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 (1 PI mono, 1 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 one 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

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP3340

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 (5 semen and 3 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 five 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 PIM) 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 2768 ng/mL and 1836 ng/mL respectively) exceeded the DRV and ATV protein adjusted MIC_{50} (Figure 1). PI concentration in RMS showed higher variability compared to 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 MIC_{50} in CVS in the study (38214 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 MIC_{50}). The median time between last ART dose and sub-study samples collection was 12.2 hours (IQR 3.8; 14.9) in participants taking DRV and 15.7 hours (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 monotherapy, which is consistent with previous reports [10,11]. Indeed, the overall prevalence of detectable HIV-RNA (below the level 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 cervico-vaginal secretions and plasma had very low viral load in both compartments (i.e. <200 copies/mL), but it was somewhat

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP3340

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].

Anti-retroviral drug penetration in the genital tract seems to be drug rather than class dependent, but overall, PI tend to reach lower concentrations than other anti-retroviral 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 MIC_{50} 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 randomised 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 STIs prevalence we identified compared with other similar populations [19] and this may also limit the generalisability of the findings. However, recent data suggest that rectal bacterial STI are 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 monotherapy was high (32%). This peaked at month nine, declining subsequently and stabilising at a much lower level by month 18 [21]. It is therefore possible that patients at highest risk of genital compartment rebound on PI monotherapy are likely to have been re-suppressed by re-induction 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 relative 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 monotherapy is unlikely to lead to ongoing sexual transmission. This may also apply to PI-based dual therapy combinations.

Acknowledgements

We thank all the patients and staff from all the centres participating in the PIVOT Genital secretions sub-study.

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP3340

The PIVOT CNS sub-study Team are:

Participating Sites

Elton John Centre, Brighton: M Fisher, A Clarke, W Hadley, D Stacey. Mortimer Market Centre, London: L Haddow, I Williams, N De Esteban, P Pellegrino, A Arenas-Pinto. Royal Liverpool University Hospital, Liverpool: N Beeching, R Tamaklo, P Davies. St James' Hospital, Leeds: J. J Minton, J Calderwood. Royal Infirmary, Manchester, UK: V Lee, K Patel, R Howe. Royal Berkshire Hospital, Reading: F Chen, R Wilson, E Green, J Masterson. Avenue House Clinic, Eastbourne: K Aderogba, M Jones. Derriford Hospital, Plymouth: Z Warwick, S Price, S Read. North Middlesex University Hospital: J Ainsworth, A Waters. Harrogate District Hospital: F Martin, C Bennett. King's College Hospital, London: F Post, L Campbell, E Wandolo. York Teaching Hospital: C Lacey, S Douglas, S Russell-Sharpe, C Brewer.

MRC Clinical Trials Unit at UCL

NI Paton, Ao Arenas-Pinto, W Stöhr, K Scott, D Dunn, K Sanders.

PIVOT Trial Steering Committee

A Freedman (Chair), B Cromarty, D Mercey, S Fidler, E Torok, A Babiker, B Gazzard, C Orkin, N Paton.

PIVOT Data Monitoring Committee

T Peto (Chair), D Lalloo, A Phillips and R James.

Disclosure statement

This analysis was funded by an unconditional scientific grant from Janssen Cilag Ltd. PIVOT was funded by the National Institute for Health Research Health Technology Assessment programme (project number 06/403/90). The views and opinions expressed herein are those of the authors and do not necessarily reflect those of the HTA programme, NIHR, NHS or the Department of Health. The authors declare no competing interests.

References

1. Paton NI, Stohr W, Arenas-Pinto A, *et al*. Protease inhibitor monotherapy for long-term management of HIV infection: a randomised, controlled, open-label, non-inferiority trial. *Lancet HIV* 2015; **2:**e417–e426.

2. Mathis S, Khanlari B, Pulido F, *et al*. Effectiveness of protease inhibitor monotherapy versus combination antiretroviral maintenance therapy: a meta-analysis. *PLoS One* 2011; **6:**e22003.

3. Arribas JR, Horban A, Gerstoft J, *et al*. The MONET trial: darunavir/ritonavir with or without nucleoside analogues, for patients with HIV RNA below 50 copies/ml. *AIDS* 2010; **24:**223–230.

4. Solas C, Lafeuillade A, Halfon P, Chadapaud S, Hittinger G, Lacarelle B. Discrepancies between protease inhibitor concentrations and viral load in reservoirs and sanctuary sites in human immunodeficiency virus-infected patients. *Antimicrob Agents Chemother* 2003; **47:**238–243.

5. van Leeuwen E, Ter Heine R, van der Veen F, Repping S, Beijnen JH, Prins JM. Penetration of atazanavir in seminal plasma of men infected with human immunodeficiency virus type 1. *Antimicrob Agents Chemother* 2007; **51:**335–337.

6. Kwara A, Delong A, Rezk N, *et al*. Antiretroviral drug concentrations and HIV RNA in the genital tract of HIV-infected women receiving long-term highly active antiretroviral therapy. *Clin Infect Dis* 2008; **46:**719–725.

7. Min SS, Corbett AH, Rezk N, *et al*. Protease inhibitor and nonnucleoside reverse transcriptase inhibitor concentrations in the genital tract of HIV-1-infected women. *J Acquir Immune Defic Syndr* 2004; **37:**1577–1580.

8. Paton NI, Stohr W, Oddershede L, *et al*. The Protease Inhibitor Monotherapy Versus Ongoing Triple Therapy (PIVOT) trial: a randomised controlled trial of a protease inhibitor monotherapy

strategy for long-term management of human immunodeficiency virus infection. *Health Technol Assess* 2016; **20:**1–158.

9. Else L, Watson V, Tjia J, *et al*. Validation of a rapid and sensitive high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) assay for the simultaneous determination of existing and new antiretroviral compounds. *J Chromatogr B Analyt Technol Biomed Life Sci* 2010; **878:**1455–1465.

10. Bunupuradah T, Bowonwattanuwong C, Jirajariyavej S, *et al*. HIV-1 genital shedding in HIV-infected patients randomized to second-line lopinavir/ritonavir monotherapy versus tenofovir/lamivudine/lopinavir/ritonavir. *Antivir Ther* 2014; **19:**579–586.

11. Torres-Cornejo A, BenMarzouk-Hidalgo OJ, Viciana P, Sanchez B, Lopez-Ruz MA, Lopez-Cortes LF, et al. Protease inhibitor monotherapy is effective in controlling human immunodeficiency virus 1 shedding in the male genital tract. Clin Microbiol Infect. 2016;22 [1]:98 e7- e10.

12. Taylor S, Sadiq ST, Weller I, *et al*. Drug-resistant HIV-1 in the semen of men receiving antiretroviral therapy with acute sexually transmitted infections. *Antivir Ther* 2003; **8:**479–483.

13. Johnson LF, Lewis DA. The effect of genital tract infections on HIV-1 shedding in the genital tract: a systematic review and meta-analysis. *Sex Transm Dis* 2008; **35:**946–959.

14. Grignolo S, Bruzzone B, Gabbi L, *et al*. Vaginal HIV-1 shedding among HIV-1 infected women in the current era of combined antiretroviral therapy: A cross sectional study. *Virulence* 2017; **8:**101–108.

15. Else LJ, Taylor S, Back DJ, Khoo SH. Pharmacokinetics of antiretroviral drugs in anatomical sanctuary sites: the male and female genital tract. *Antivir Ther* 2011; **16:**1149–1167.

16. Cottrell ML, Srinivas N, Kashuba AD. Pharmacokinetics of antiretrovirals in mucosal tissue. *Expert Opin Drug Metab Toxicol* 2015; **11:**893–905.

17. Rittweger M, Arasteh K. Clinical pharmacokinetics of darunavir. *Clin Pharmacokinet* 2007; **46:**739–756.

18. Brown KC, Patterson KB, Jennings SH, *et al*. Single- and multiple-dose pharmacokinetics of darunavir plus ritonavir and etravirine in semen and rectal tissue of HIV-negative men. *J Acquir Immune Defic Syndr* 2012; **61:**138–144.

19. Kalichman SC, Pellowski J, Turner C. Prevalence of sexually transmitted co-infections in people living with HIV/AIDS: systematic review with implications for using HIV treatments for prevention. *Sex Transm Infect* 2011; **87:**183–190.

20. Storim J, Verheyen J, Wolff E, *et al*. Antiretroviral therapy suppresses rectal HIV-RNA shedding despite inflammation in MSM with rectal C. trachomatis and N. gonorrhoeae infections-a cross-sectional, single-center study. *Sex Transm Infect* 2018.

21. Stohr W, Dunn DT, Arenas-Pinto A, *et al*. Factors associated with virological rebound in HIV-infected patients receiving protease inhibitor monotherapy. *AIDS* 2016; **30:**2617–2624.

Figure

Figure 1. Protease Inhibitor concentration (PI-mono and triple therapy groups combined)

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP3340

Table. Study population

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

PI-mono: PI monotherapy; OTT: Ongoing triple therapy

Figure. Protease Inhibitor concentration (PI-mono and triple therapy groups combined)