INTERNATIONAL MEDICAL PRESS

Antiviral Therapy

Pharmacokinetics of raltegravir in the semen of HIV-infected men

Tony Antoniou, Mona R Loutfy, Jason Brunetta, Graham Smith, Roberta Halpenny, Charles la Porte

Antiviral Therapy 2014; 10.3851/IMP2750

Submission date 1st August 2013
Acceptance date 27th January 2014
Publication date 12th February 2014

ISSN 1359-6535

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP2750

Short communication

Pharmacokinetics of raltegravir in the semen of HIV-infected men

Tony Antoniou[1,2], Mona R Loutfy[3,4,5,6], Jason Brunetta[6], Graham Smith[6], Roberta Halpenny[6], Charles la Porte[7,8]*

[1]Department of Family and Community Medicine, St. Michael's Hospital, Toronto, ON, Canada

[2]Leslie Dan Faculty of Pharmacy, University of Toronto, Toronto, ON, Canada

[3]Institute of Health Policy, Management and Evaluation, University of Toronto, Toronto, ON, Canada

[4]Department of Medicine, University of Toronto, Toronto, ON, Canada

[5]Women's College Research Institute, Women's College Hospital, University of Toronto, Toronto, ON, Canada

[6]Maple Leaf Medical Clinic, Toronto, ON, Canada

[7]Ottawa Hospital Research Institute, Ottawa, ON, Canada

[8]Janssen Cilag BV, Tilburg, the Netherlands

*Corresponding author e-mail: tantoniou@smh.toronto.on.ca

Abstract

Background: We sought to determine the pharmacokinetic disposition of raltegravir in the blood and seminal plasma of HIV-infected men.

Methods: We conducted a pharmacokinetic study using a staggered sampling approach. 16 HIV-infected men receiving raltegravir-based therapy were recruited into the study. Each participant provided six blood plasma and six seminal plasma samples for quantification of drug concentrations in both compartments. Blood and semen samples were obtained within 1 hour of each other, and were collected prior to the morning dose, and at 1, 2, 4, 8 and 12 hours post-ingestion. Drug concentrations were determined by liquid chromatography tandem mass spectrometry.

Results: A total of 96 semen samples and 96 blood samples were obtained from all participants during the study period. The median age and baseline CD4+ cell count of the study participants were 48 years (interquartile range 42 to 53 years) and 450 cells/mm^3 (interquartile range 289 cells/mm^3 to 585 cells/mm^3). Virologic suppression to < 50 copies/mL had been maintained for a median of 21 months (interquartile range 7 to 35 months) at the time of study enrolment. The median seminal plasma to blood plasma ratios and $AUC_{0\text{-}12h}$ seminal plasma to blood plasma ratios of raltegravir were 3.25 (interquartile range 1.46 to 5.37) and 2.26 (interquartile range 1.05 to 4.45), respectively.

Conclusions: Concentrations of raltegravir in seminal plasma are several fold-higher than those attained in blood plasma and those required to inhibit viral

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP2750

replication in this compartment. Further research examining the therapeutic and prophylactic implications of these findings is warranted.

Accepted 27 January 2014, published online 12 February 2014

Running head: Raltegravir levels in semen

Introduction

Although combination antiretroviral therapy (cART) is generally associated with virologic suppression in both the blood and seminal plasma of HIV-infected men, several studies have described discordant virologic responses and genotypic drug sensitivities between these compartments [1–6]. These divergent patterns are observed in 7-15% of patients receiving cART, and are most likely attributable to different degrees of antiretroviral distribution into the male genital tract [1–6]. In this scenario, suboptimal seminal plasma concentrations of one or more drugs may predispose individuals to ongoing viral replication and the emergence of drug-resistant variants within this compartment despite full virologic suppression in the blood [1–6]. In addition, challenges associated with the development of an effective vaccine against HIV have heightened interest in examining the efficacy of antiretroviral drugs for the prevention of HIV acquisition [7]. Although the physicochemical and pharmacokinetic drug properties associated with effective pre-exposure prophyalxis against HIV require further delineation, accumulation in gential tissues is likely a mandatory prerequisite for antiretrovirals that will be used for this purpose [7]. However, because of logistical and practical challenges associated with collecting multiple specimens from participants, many studies quantifying the concentration of antiretrovirals in semen use a single matched time point to derive seminal plasma to blood plasma ratios (SP:BP) of these drugs [8]. Consequently, data regarding additional salient markers of antretroviral disposition within this compartment are frequently unavailable.

Raltegravir is a potent integrase strand transfer inhibitor that is used to treat both antiretroviral naïve and treatment experienced patients [9]. With the exception of our previously reported study examining the pharmacokinetics of maraviroc, raltegravir, darunavir and etravirine in a sample of 10 HIV-positive men, there are no data characterizing the disposition of raltegravir in the seminal compartment of men with HIV over the entire dosing interval [10]. Other published studies assessing the distribution of raltegravir into the male genital tract have utilized single semen samples from HIV-infected men or healthy volunteers [11–14]. In light of the gaps in the literature, we undertook a separate study to characterize the pharmacokinetic disposition of raltegravir over a 12-hour dosing interval in the semen of HIV-infected men.

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP2750

Methods

Study Design

We prospectively recruited 16 HIV-infected men aged 18 years and older who had been receiving raltegravir-based cART for a minimum of ninety days. Additional eligibility criteria included having an undetectable viral load (< 50 copies/mL) for a minimum of one month, ability to provide written informed consent, and no active illness or comorbidity, including acute renal or hepatic disease. We excluded patients who were suspected of non-adherence to their prescribed regimen and who were expected to have difficulty adhering to the study protocol for any reason. We obtained written informed consent from all participants of the study.

We used a staggered sampling approach in which semen samples were produced by participants over several days at different sampling times relative to the morning dose of raltegravir. The study protocol did not specify any food requirements, restrictions or calorie content with respect to the morning dose of raltegravir. Semen samples were collected 30 minutes to one hour prior to the morning dose of raltegravir (day 1), and then at hours 1, 2, 4, 8 and 10 to 12 post-drug ingestion on days two through six. We collected corresponding blood samples within one hour of the semen sample.

Specimen processing and analysis

Semen and blood samples were centrifuged without delay, and isolated seminal and blood plasma were aliquoted and stored at – 80ºC until analyzed.

Plasma concentrations (both in blood and semen) of raltegravir were determined by using a validated liquid chromatography mass spectrometry/mass spectrometry (LC/MS/MS) method. 200 µL blood plasma (100 µL seminal plasma) was spiked with 6,7-dimethyl-2,3-di(2-pyridyl)-quinoxaline (Aldrich, Milwaukee, WI, USA) as internal standard (IS) and subjected to protein precipitation with acetonitrile (1:3) followed by centrifugation at 5,000 X g for 5 minutes. For the preparation of the seminal calibration curve drug free seminal plasma was used, in the same way drug free blood plasma was used for the preparation of the blood plasma calibration curve. The LC-MS/MS system consisted of a HP1100 LC system (Agilent Technologies, Wilmington, DE, USA) with a SupelcosilTM ABZ+ [15 cm x 4.6mm, 3 µm] C18 column (Supelco, Bellefonte, PA, USA) coupled to an API-2000 mass spectrometer (AB/MDS/Sciex, Concord, ON, Canada) with a turbo ion spray source. LC was performed at 40 °C with a gradient elution of acetonitrile-0.1% (v/v) formic acid in water at a flow rate of 1ml/min. MS was quantified using electrospray multiple reaction monitoring(MRM) in positive mode and the MRM transitions were m/z 445 to109 and m/z 313 to 246.4 for raltegravir and the IS, respectively. The absolute recoveries were 93-100% in blood and 95-110% in semen. Validation results displayed that raltegravir was stable for 24 hours at 4 °C after sample preparation and during 3 freeze-thaw cycles.The effective linear range was 22.5-4500ng/mL in both blood and semen. Interbatch precision (CV%) varied between 6.1 and 12.7% in

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP2750

blood and 2.1-13% in semen, and intrabatch accuracy varied between 98.8 and 102.8% in blood and 104.4 and 109.7% in semen.

Data analyses

We used non-compartmental analyses to determine the pharmacokinetic parameters of raltegravir in seminal and blood plasma. Specifically, we used the exact sample collection and data observation times to determine the maximum (C_{max}), minimum concentrations (C_{min}) and time to maximum concentration (T_{max}) and area under the curve by using the trapezoidal rule from zero to twelve hours ($AUC_{0\text{-}12h}$) in both compartments, and also determined the coefficient of variation of SP:BP ratios. Pharmacokinetic parameters are presented using medians and interquartile ranges (IQR). We used published estimates of raltegravir IC_{95} for HIV-1 to facilitate interpretation of our results [15].

Ethics approval

We obtained ethics approval for this study from Institutional Review Board Services and the Ottawa Hospital Research Ethics Board.

Results

Study population

The median age and baseline CD4+ cell count of the 16 study participants were 48 years (interquartile range 42 to 53 years) and 450 cells/mm^3 (interquartile range 289 cells/mm^3 to 585 cells/mm^3). Virologic suppression to < 50 copies/mL had been maintained for a median of 21 months (interquartile range 7 to 35 months) at the time of study enrolment. Concomitant therapy with darunavir/ritonavir, etravirine and tenofovir was received by nine, seven and thirteen patients, respectively.

Pharmacokinetic analyses

A total of 96 semen samples and 96 blood samples were obtained from all participants during the study period. Paired seminal and blood plasma concentrations of raltegravir were determined in all participants.

Pharmacokinetic parameters of raltegravir in blood plasma and seminal plasma are shown in Figure 1 and Table 1. The median raltegravir C_{min}, C_{max} and $AUC_{0\text{-}12h}$ in seminal plasma were 0.18 mg/L (interquartile range 0.13 to 0.24 mg/L), 1.91 mg/L (interquartile range 1.46 to 4.33 mg/L) and 6.83 h*mg/L (interquartile range 5.24 to 13.47 h*mg/L), respectively. The median raltegravir SP:BP and $AUC_{0\text{-}12h}$ SP:BP ratios were 3.25 (interquartile range 1.46 to 5.37) and 2.26 (interquartile range 1.05 to 4.45), respectively, with coefficients of variation for each parameter of 86%.

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP2750

Discussion

The results of our study demonstrate a high and variable degree of raltegravir penetration into the male genital tract over a twelve-hour dosing interval. Furthermore, seminal plasma trough concentrations of raltegravir exceeded the published IC_{95} for wild-type HIV-1 (0.0146 mg/L) by 12.3-fold, suggesting that that these levels would be adequate for viral suppression within the male genital tract. These results were qualitatively similar to those we observed in our smaller study examining the pharmacokinetics of raltegravir in the semen of 10 HIV-infected men receiving maraviroc-based antiretroviral therapy [10]. We speculate that, by virtue of being slightly lipophilic and 83% bound to blood plasma proteins, raltegravir distributes into and accumulates within seminal plasma by passive diffusion [11,16]. In addition, although the effects of genital tract drug transporters on antiretroviral levels within this compartment are poorly characterized, it is possible that protease-inhibitor mediated blockade of p-glycoprotein prevents active transport of raltegravir out of this compartment, as many patients were concomitantly on darunavir/ritonavir [17,18]. However, our sample size prevented us from making formal comparisons between patients receiving darunavir/ritonavir with those not receiving these drugs, and therefore further research is required to examine this hypothesis.

Our study builds upon earlier research in which SP:BP ratios for raltegravir were derived from single matched time points. Specifically, median SP:BP ratios for raltegravir ranged from 1.42 to 4.9 in previous studies of HIV-positive men [11–13]. In a study of eight healthy volunteers the SP:BP ratio for raltegravir was four-times greater at the end of the dosing interval relative to the 2 to 4 hour period following drug intake (6.45 versus 1.62, respectively) supporting a gradual accumulation of drug within the male genital tract over time [14]. However, unlike these studies, we used a staggered sampling approach and were therefore able to generate seminal plasma area under the concentration time curves for raltegravir and provide an evaluation of the seminal plasma pharmacokinetics of raltegravir over the entire dosing interval in HIV-infected men receiving this drug.

Several limitations of our work merit emphasis, including the small sample size and the inability to directly measure unbound antiretroviral drug concentrations in seminal plasma. In addition, we could not estimate the unbound trough concentration of raltegravir because the degree of protein binding of this drug in seminal plasma is unknown. However, these limitations are common to pharmacokinetic studies evaluating drug concentrations in semen [19]. Finally, we did not specify any food requirements with the morning dose of raltegravir, thereby introducing a potential source of intra-subject variability in raltegravir pharmacokinetics [20]. However, this is consistent with dosing guidelines for raltegravir, and existing data suggest that there are no clinically important effects of food on the disposition of raltegravir [20,21].

In conclusion, our study demonstrates that raltegravir attains semen concentrations that are several fold higher than those observed in the blood plasma of HIV-infected men. Further research examining the role of raltegravir in the primary and secondary prevention of HIV is warranted.

Publication: Antiviral Therapy; Type: Short communication
DOI: 10.3851/IMP2750

Acknowledgements

We would like to thank all patients involved in this study and Tigist Kidane for her technical assistance with this study.

Funding/Support

This work was supported by an unrestricted research grant from Merck Canada Inc. The sponsors had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript. The opinions, results and conclusions reported in this paper are those of the authors and are independent from the funding source.

Tony Antoniou is supported by a post-doctoral fellowship from the Ontario HIV Treatment Network.

Disclosure Statement

During the past five years, Tony Antoniou has received unrestricted research grants from Glaxo-Pfizer for a different study. Mona Loutfy has received unrestricted research grants for other projects from, and has acted as a speaker and advisor for, Abbott Canada, Abbvie Canada, Merck Frosst, Pfizer, Bristol-Myers Squibb, Tibotec, Johnson & Johnson Inc., Boehringer Ingelheim, and GlaxoSmithKline, ViiV Healthcare and Gilead Sciences. Jason Brunetta has received honoraria for consulting and advisory work from Abbott, ViiV, Tibotec, Merck, Eli Lilly, and Gilead. Graham Smith has received speaker fees and advisory board fees from Merck, Abbott, Viiv and Tibotec/Jansen. Charles la Porte has received grants or research support from, or served as a consultant, advisor or speaker for Abbott Laboratories, Bristol-Myers Squibb, Merck, Roche, Boehringer Ingelheim, Pfizer and Tibotec, and is currently employed by Janssen Cilag BV. Roberta Halpenny has no conflicts of interest to declare.

References

1. Lafeuillade A, Solas C, Halfon P, *et al*. Differences in the detection of three HIV-1 protease inhibitors in non-blood compartments: clinical correlations. *HIV Clin Trials* 2002; **3:**27–35.

2. Leruez-Ville M, Dulioust E, Costabliola D, *et al*. Decrease in HIV-1 seminal shedding in men receiving highly active antiretroviral therapy: an 18 month longitudinal study (ANRS EP012). *AIDS* 2012; **16:**486–488.

3. Lafeuillade A, Solas C, Chadapaud S, *et al*. HIV-1 RNA levels, resistance, and drug diffusion in semen vs. blood in patients receiving a lopinavir-containing regimen. *J Acquir Immune Defic Syndr* 2003; **32:**462–464.

4. Varoso PF, Schechter M, Gupta P, *et al*. Adherence to antiretroviral therapy and persistence of HIV RNA in semen. *J Acquir Immune Defic Syndr* 2003; **32:**435–440.

5. Bujan L, Daudin M, Matsuda T, *et al*. Factors of intermittent HIV-1 excretion in semen and efficiency of sperm processing in obtaining spermatozoa without HIV-1 genomes. *AIDS* 2004; **18:**757–766.

6. Lorello G, la Porte C, Pilon R, *et al*. Discordance in HIV-1 viral loads and antiretroviral drug concentrations comparing semen and blood plasma. *HIV Med* 2009; **10:**548–554.

7. Heneine W, Kashuba A. HIV prevention by oral preexposure prophylaxis. *Cold Spring Harb Perspect Med* 2012; **2:**a007419.

8. Else LJ, Taylor S, Back DJ, *et al*. Pharmacokinetics of antiretroviral drugs in anatomical sanctuary site: the male and female genital tract. *Antivir Ther* 2011; 1149–1167.

9. Rokas KE, Bookstaber PE, Shamroe CL, *et al*. Role of raltegravir in HIV-1 management. *Ann Pharmacother* 2012; **46:**578–589.

10. Antoniou T, Hasan S, Loutfy MR, *et al*. Pharmacokinetics of maraviroc, raltegravir, darunavir and etravirine in the semen of HIV-infected men. *J Acquir Immune Defic Syndr* 2013; **62:**e58–e60.

11. Barau C, Delaugerre C, Braun J, *et al*. High concentration of raltegravir in semen of HIV-infected men: results from a substudy of the EASIER-ANRS 138 trial. *Antimicrob Agents Chemother* 2010; **54:**937–939.

12. Carey D, Pet SL, Bloch M, *et al*. A randomized study of pharmacokinetics, efficacy, and safety of 2 raltegravir plus atazanavir strategies in ART-treated patients. *J Acquir Immune Defic Syndr* 2012; **60:**143–149.

13. Osborne BJW, Sheth PM, Yi TJ, *et al*. Impact of antiretroviral therapy duration and intensification on isolated shedding of HIV-1 RNA in semen. *J Infect Dis* 2013; **207:**1226–1234.

14. Calcagno A, Bonora S, D'Avolio A, *et al*. Raltegravir penetration in seminal plasma of healthy volunteers. *Antimicrob Agents Chemother* 2010; **54:**2744–2745.

15. Iwamoto M, Wenning LA, Petry AS, *et al*. Safety, tolerability, and pharmacokinetics of raltegravir after single and multiple doses in healthy subjects. *Clin Pharmacol Ther* 2008; **83:**293–299.

16. Temesgen Z, Siraj DS. Raltegravir: first in class HIV integrase inhibitor. *Ther Clin Risk Manag* 2008; **4:**493–500.

17. Kis O, Robillard K, Chan GN, *et al*. The complexities of antiretroviral drug-drug interactions: role of ABC and SLC transporters. *Trends Pharmacol Sci* 2010; **31:**22–35.

18. Griffin L, Annaert P, Brouwer KL. Influence of drug transport proteins on the pharmacokinetics and drug interactions of HIV protease inhibitors. *J Pharm Sci* 2011; **100:**3636–3654.

19. Avery LB, Rakshi RP, Cao YJ, *et al*. The male genital tract is not a pharmacological sanctuary from efaviranz. *Clin Pharmacol Ther* 2011; **90:**151–156.

20. Brainard DM, Friedman EJ, Jin B, *et al*. Effect of low-, moderate-, and high-fat meals on raltegravir pharmacokinetics. *J Clin Pharmacol* 2011; **51:**422–427.

21. Merck Frosst Canada Ltd. Isentress (raltegravir) Prescribing Information. Kirkland, QC

.

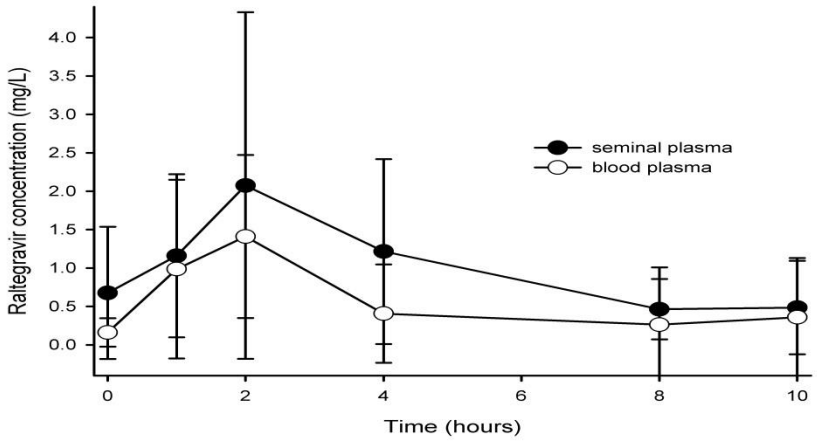
Figure Legend

Figure 1: Concentrations (median, IQR) of raltegravir in blood and seminal plasma (n=16)

Table 1: Antiretroviral pharmacokinetic parameters of raltegravir in blood and seminal plasma (n=16)

Pharmacokinetic parameters (median, IQR)	**Blood Plasma**	**Seminal Plasma**
C_{min} (mg/L)	0.04 (0.03 – 0.05)	0.18 (0.13 – 0.05)
C_{max} (mg/L)	2.04 (0.84 – 2.83)	1.91 (1.46 – 4.33)
T_{max} (hours)	2.00 (2.00 – 4.00)	2.00 (2.00 – 4.00)
$AUC_{0\text{-}12h}$ (hours * mg/L)	5.36 (2.58 – 7.40)	6.83 (5.24 – 13.47)

*C_{min}, minimum drug concentration; C_{max}, maximum drug concentration; T_{max} time of maximum drug concentration; $AUC_{0\text{-}12h}$, area under the concentration versus time curve within the 12-hour dosing interval


Raltegravir concentration (mg/L)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
Time (hours)
seminal plasma
blood plasma