#854

Influence of Raltegravir versus Boosted Atazanavir Regimens on Cervical Immune Reconstitution

Amie L. Meditz[1], Claire Palmer[1], Julie Predhomme[1], Kristina Searls[1], Becky Kerr[1], Patricia Caraway[2], Edward M. Gardner[1,2] Sam Mawhinney[1], Peter L. Anderson[1*]

[1]University of Colorado, Aurora, Colorado, USA; [2]Denver Public Health, Denver, CO, USA

Abstract

Background: A significant amount of HIV-related morbidity has been attributable to genital tract disease in women. It is established that tenofovir (TDF), emtricitabine (FTC), and raltegravir (RAL) have high genital concentrations while atazanavir/ritonavir (ATZ) has more modest levels. We examine if RAL vs ATZ-based regimen is superior with respect to improved cervical immune reconstitution and less immune activation in HIV+ women.

Methods: Peripheral blood (PB), cervical brush and lavage fluid (CVL), and cervical biopsies were collected from HIV+ women receiving TDF/FTC and either ATZ (n=19) or RAL (n=14). A history of CD4+ T-cells/mm³ (CD4) ≥ 300, and HIV RNA <48 copies/mL for a minimum of 6 months was required for entry. Cervical cells were analyzed by flow cytometry to determine %HLA-DR+CD38+(DR+38+) on CD4+ and CD8+T-cells. Sections of cervical biopsies were stained by immunohistochemistry and frequencies of CD3+CD4+, and CD3+CD8+cells per mm² of tissue were determined using quantitative image analysis. RAL and ATZ trough concentrations in plasma and CVL were quantified with validated assays. T-tests, Fisher's exact and linear regressions were used with log transformations as appropriate. Data are expressed as means or geometric means (CI).

Results: RAL and ATZ groups did not differ by age (46 vs 43 years, p=0.5), race (p=1.0), CD4 nadir (309 vs 340, p=0.65), current CD4 (663 vs 758, p=0.4) or PB CD4:CD8 ratio (1.0 (0.84, 1.18) vs 1.03 (0.78, 1.37), p=0.8). Years since HIV diagnosis and years on current ARV was significantly lower in women taking RAL vs ATZ (8 vs 13; p=0.03 and 2 vs 5; p=0.01, respectively). Plasma RAL vs ATZ levels were 121 (44, 335) vs 620 (419, 918) ng/mL. CVL levels for RAL vs ATZ were 9 (4, 23) vs 4 (2, 8) ng/mL. Genital:plasma (G:P) concentration ratio was significantly higher for RAL (0.078 (0.034, 0.18)) vs ATZ (0.006 (0.004,0.011), p<0.001). Drug concentrations in plasma correlated with those in CVL (RAL p=0.63, p=0.02; ATZ p=0.54, p=0.03). RAL and ATZ groups did not differ by cervical %DR+38+CD4+T-cells (20 vs 16%, 0=0.4) or %DR+38+CD8+T-cells (23 vs 21%, p=0.7). Cervical CD4:CD8 T-cell ratio in RAL group 0.46 (0.33, 0.63) was not significantly different than ATZ group (0.55 (0.36, 0.82); p=0.5). In HIV- women, the CD4:CD8 T-cell ratio has been reported as 4:1. CD4:CD8 T-cell ratio was significantly higher in PB than cervical tissue in both groups (p<0.002). After adjusting for time on ARV, log(G:P drug ratio) was not a significant predictor of cervical CD4:CD8+ T-cell ratio or immune activation (p≥0.2).

Conclusions: Low cervical tissue CD4:CD8 ratio persists after years of ARV. Despite higher genital concentrations of RAL compared to ATZ, there was no significant difference in cervical CD4:CD8 ratio or immune activation between women on these regimens.

Background

A significant amount of HIV-related morbidity is attributable to genital tract disease in women [1-3]. It is established that the antiretroviral drugs tenofovir (TDF), emtricitabine (FTC), and raltegravir (RAL) achieve high genital concentrations while atazanavir/ritonavir (ATZ) attains more modest levels [4,5].

The purpose of this work was to evaluate if RAL vs ATZ-based regimen is superior with respect to improved cervical immune reconstitution and less immune activation in cervical tissue from HIV-seropositive women.

Methods

Study Subjects: Samples were collected over a 2 week time interval from HIV-1 infected women receiving TDF/FTC and either RAL or ATZ/r. Peripheral blood mononuclear cells (PBMC) and cervical-vaginal lavage (CVL) and brush samples were collected at week one and PBMC, CVL, and cervical biopsies were collected at week two. Entry criteria for study included a history of CD4+ T cells/mm³ ≥300 and HIV RNA copies/mL <48 for a minimum of 6 months on the regimen of interest. Informed consent was obtained in accordance with the University of Colorado IRB.

Clinical Specimens: Peripheral blood CD4⁺ T-cell counts were determined by flow cytometry; plasma HIV-1 RNA concentration was measured by Roche COBAS TaqMan 96 HIV-1 test; Herpes Simplex Virus measured by PCR; Human Papilloma Virus (HPV) was measured by DNA testing; direct microscopy was used to evaluate for clue cells, Trichomonas, and Candida; nucleic acid amplification was used to detect Chlamydia trachomatis and Neisseria gonorrhea; and treponemal antibodies were used to screen for syphilis. All assays were performed in the University of Colorado Hospital Clinical Laboratory (Aurora, CO).

Quantification of CD3, CD4, and CD8 in Cervical Biopsies: Snap frozen cervical biopsy specimens were sectioned into 4 µm sections, and an average of 8 sections each approximately 120 µm apart were evaluated for each subject. Sections were immunostained with fluorescent antibodies to CD3, CD4, and CD8, and visualized by immunofluorescent microscopy. Numbers of CD3+CD4+ and CD3+CD8 cells per mm² of tissue were determined using visual inspection and quantitative image analysis and used to calculate the cervical CD4:CD8 ratio.

Flow Cytometry Analysis: Whole blood and cervical cells were stained in real time with antibodies to CD3-PEcy5 (BD Biosciences, San Jose, CA), CD4-APC-H7, CD8-Alexa Fluor 405, CD38-FITC (Invitrogen Life Science), HLA-DR-APC, CCR5-PE (BD Biosciences, CCR5 with known 1:1 PE:antibody ratio), as previously described [6]

Quantification of RAL & ATZ : RAL and ATZ trough concentrations in plasma and CVL were quantified using a validated LC-MS-MS methodology, as previously described [7,8].

Statistical Analysis: T-tests, Fisher's exact and linear regressions were used with log transformations as appropriate. Continuous outcomes are summarized using means or geometric means (CI). (R version 2.13.2, http://cran.r-project.org/)

Results

TABLE 1: Demographic and Clinical Characteristics (95% CI)

	RAL (n=14)	ATZ (n=19)	P-value
Age, Years, mean	44 (38, 50)	43 (38, 48)	0.70
Race, No. (%)			0.72
Black	4 (29%)	7 (37%)	
Non-Black	10 (71%)	12 (63%)	
Years on Current Antiretroviral Regimen	2 (1, 4)	5 (3, 6)	0.02
Years Since HIV Diagnosis	8 (5, 11)	13 (9,16)	0.04
Historic Nadir CD4+T cells/mm², mean*	275 (192, 357)	340 (246, 435)	0.27
CD4+ T cells/mm², Geo. mean	663 (495, 890)	758 (652, 882)	0.40
CD8+ T cells/mm², Geo. mean**	632 (464,863)	759 (625, 921)	0.30
HPV Positive, No. (%)	2 (14%)	4 (21%)	1.0
Clue Cells, No. (%)			0.42
None	12 (86%)	13 (68%)	
Present	2 (14%)	6 (32%)	
Plasma Drug Trough Concentration, ng/mL, Geo. mean	121(44, 335)	656 (445, 966)	
Cervical Drug Concentration, ng/0.5 mL CVL, Geo. mean**	9.45 (3.95, 22.64)	4.34 (2.36, 7.97)	

*ATZ, n=17; **ATZ, n=18; *All subjects had negative gonorrhea, chlamydia, syphilis, and HSV PCR testing, and urine β HCG at time of sample collection .*

FIG. 1: RAL (A) and ATZ (B) plasma levels were significantly associated with cervical drug levels. Genital to plasma drug ratios (C) were significantly higher in women receiving RAL compared to ATZ (lines indicate geo. mean).

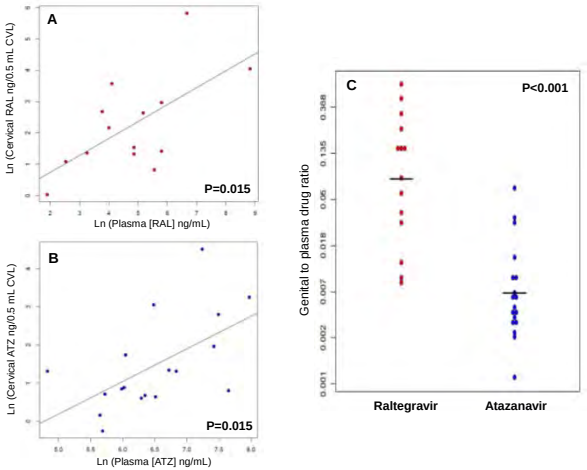


TABLE 2: Peripheral blood and cervical CCR5 expression and HLA-DR and CD38 expression on CD4+ and CD8+ T cells were not different between treatment groups.

	Whole Blood			Cervical Brush		
	Raltegravir (n=14)	Atazanavir (n=19)	P-value	Raltegravir (n=14)	Atazanavir (n=19)	P-value
%CCR5+CD4+ T cells	13 (8,21)	14 (11,17)	0.86	63 (56, 70)	63 (56, 71)	0.90
Density of CCR5 on CD4+ T cells (mol/cell)	1228 (1113,1355)	1267 (1133, 1417)	0.66	1794 (1520, 2117)	1768 (1491, 2097)	0.90
%CCR5+DR+38+CD4+ T cells	58 (49, 69)	63 (57, 70)	0.43	90 (83,97)	91 (86,96)	0.69
Density of CCR5 on DR+38+CD4+ T cells (mol/cell)	2085 (1630, 2669)	1772 (1504, 2089)	0.25	2031 (1674, 2465)	2166 (1799, 2609)	0.61
%DR+38+CD4+ T cells	2 (1,2)	2 (1,2)	0.87	20 (14, 29)	16 (11, 24)	0.35
%DR+38+CD8+ T cells	4 (3, 5)	5 (4, 7)	0.10	22 (17, 30)	21 (16, 27)	0.68

FIG. 2: Representative images of CD3, CD4, and CD8 staining in a cervical biopsy specimen (40X).

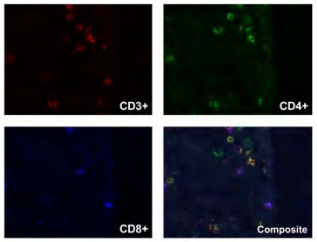


FIG. 3: There was a significant association between peripheral blood CD4:CD8 ratio and cervical CD4:CD8 ratio combining results from all women.

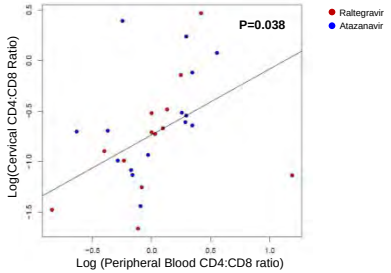


FIG. 4: Cervical CD4:CD8 T-cell ratio in RAL group geo. mean, 0.46 (95% CI, 0.33, 0.63) was not significantly different than that in the ATZ group geo. mean, 0.52 (95%CI, 0.36, 0.75). The cervical CD4:CD8 ratios in HIV+ women were substantially lower than the ratio of 4:1, previously reported as the median in HIV- women [9].

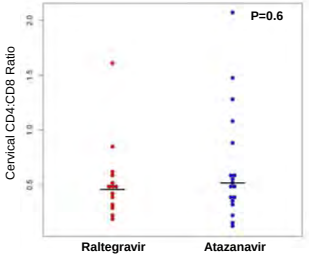
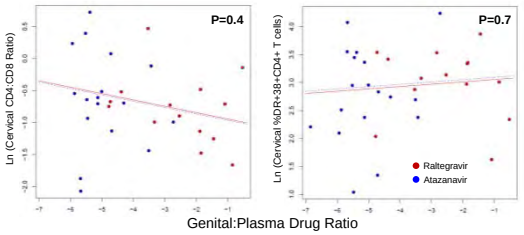


FIG. 5: Genital:Plasma drug ratio was not a significant predictor of cervical CD4:CD8+ T-cell ratio (A) or immune activation in the cervix (B) after adjusting for treatment and time on antiretroviral therapy.



Conclusions

- **Low cervical tissue CD4:CD8 ratio persists in HIV-infected women after years of ARV and may contribute to genital tract morbidity.**
- **Despite higher genital to plasma concentration ratios of RAL compared to ATZ, there was no significant difference in cervical CD4:CD8 ratio or immune activation between women taking RAL and ATZ.**
- **Lack of immunologic effect associated with increased genital drug penetration may be because drug concentrations were sufficient in all subjects to suppress virus replication.**
- **Whether differential drug compartmentalization has other biologic effects warrants further study.**

References

1. Meditz, JID, 2011,203:442.
2. Mayaud, Sex Transm Infect 2008,84:332-337.
3. Bratcher, Infect Agent Cancer 2010,5:8
4. Cohen, Ann Intern Med 2007,146:591
5. Patterson K., Pharmacokinetics of RAL in the blood plasma and genital tract in HIV+ and HIV- women. XVIII International AIDS Conference. Vienna, Austria; 2010.
6. Meditz, JAIDS 2012, 59:221
7. Kiser, Antimicrob Agents Chemother 2010,54:4999
8. Anderson, J Antimicrob Chemother 2009,64:1071
9. Nkwanyana, Immunology, 2009, 128: e746

Contact Information

Peter L. Anderson, PharmD
Associate Professor of Medicine
University of Colorado, Pharmaceutical Sciences
2850 E. Montview Blvd.,
Aurora, CO 80045
303-724-6128
Peter.Anderson@ucdenver.edu

Amie L. Meditz, M.D.
Boulder Community Hospital
1136 Alpine Ave., STE 205
Boulder, CO 80304
ameditz@bch.org

Acknowledgments

This work was supported by an investigator initiated grant from Merck # 39423. The cervical drug concentrations were analyzed by the University of North Carolina at Chapel Hill with support from the Center for AIDS Research (CFAR), an NIH funded program P30 AI50410. We wish to express our gratitude to the women who participated in this study.

University of Colorado Anschutz Medical Campus