RPV LA Does Not Inhibit Resistant HIV Transmission or Select Significant Resistance

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Abstract

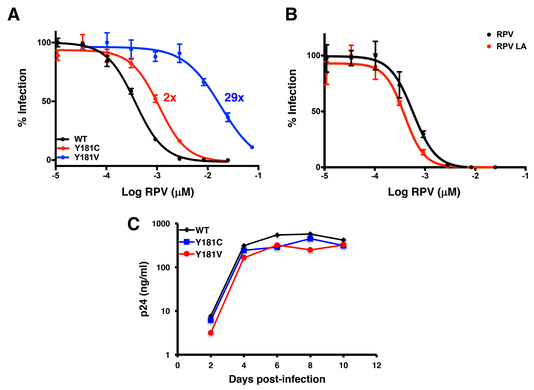
BACKGROUND: Long-acting rilpivirine (RPV LA) has been proposed for use as pre-exposure prophylaxis (PrEP) and the prevalence of transmitted RPV-resistant viruses can be relatively high in some populations. We studied how effectively RPV LA could inhibit vaginal transmission of WT HIV-1 as well as two RPV-resistant mutants.

METHODS: Plasma and female genital tract (FGT) RPV pharmacokinetics were determined after a single dose of RPV LA in female humanized BLT mice. Vaginal virus challenges of WT, Y181C, and Y181V HIV-1 were performed in untreated animals and after RPV PrEP when plasma and FGT concentrations were either at biologically relevant (low) or 7- to 9-fold higher (high) concentrations (n=6-10 per group). Quantitative RT-PCR was used measure plasma viremia in the mice until 10 weeks post-challenge. Single-genome sequencing of the reverse transcriptase coding region was performed on plasma HIV-1 RNA in mice infected during PrEP treatment.

RESULTS: Y181C HIV-1 conferred 2-fold resistance and Y181V HIV-1 conferred 30-fold resistance to RPV in vitro. High RPV concentrations delayed dissemination of a highly infectious WT HIV-1 clone ($p>0.05$) and completely inhibited Y181C HIV-1 ($p=0.02$). However, high RPV concentrations did not inhibit Y181V HIV-1. Biologically relevant RPV concentrations did not significantly inhibit vaginal transmission of WT or Y181C HIV-1. New mutations associated with low-level RPV were detected in 25% of mice infected during RPV LA PrEP usually at frequencies of <5% of HIV-1 RNA genomes. E138K and mutations that do not confer RPV resistance (M184I and V179I) were also observed in some mice. E138K and M184I previously were shown to develop via APOBEC3-mediated hypermutation.

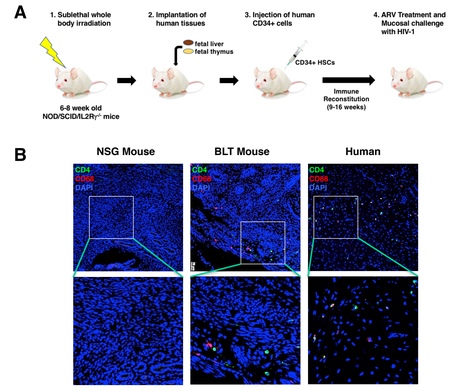
CONCLUSIONS: Biological concentrations of RPV in the genital tract and plasma were not sufficient to prevent vaginal transmission of a highly infectious HIV-1 molecular clone. While 8-fold higher RPV concentrations inhibited low RPV-resistant Y181C HIV-1, they were not sufficient to inhibit Y181V HIV-1, which has 30-fold resistance to RPV. Our data suggest that both level of resistance and infectivity affect the ability of HIV-1 to be transmitted during PrEP. HIV-1 did not develop high level or high frequency RPV resistance most mice infected during RPV LA PrEP. The impact of low frequency RPV-resistant viruses on virologic outcome during antiretroviral treatment remains to be determined.

Figure 1: Characterization of HIV-1 RT Y181 Mutants



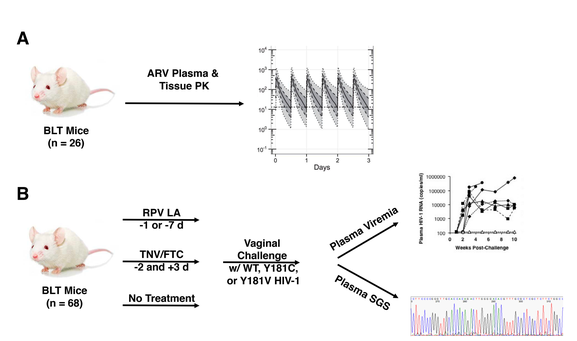
(A) Infection of WT, Y181C, and Y181V HIV-1 was measured in TZMbl cells in the presence of multiple concentrations of RPV. The results are representative of 3 independent experiments, each performed in triplicate. (B) Infection of WT HIV-1 was measured in TZMbl cells in the presence of multiple concentrations of RPV or RPV LA. (C) Replication kinetics of WT, Y181C, and Y181V HIV-1 was performed in human PBMC as measured by production of p24 over time in culture supernatants.

Figure 2: BLT Mouse Model



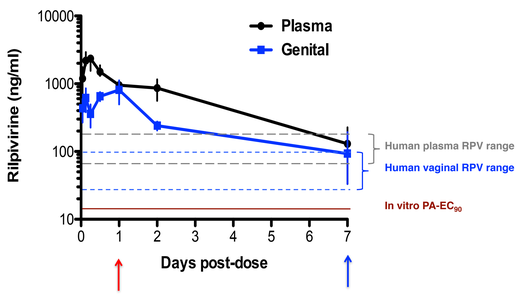
(A) Generation of humanized BLT mice. (B) Flash frozen female genital tract were obtained from a NSG mouse (the background strain used) prior to reconstitution with human cells, a BLT mouse, and cervical tissue from a human woman. Tissues were fixed, sectioned, and stained with human anti-CD4 (green) and anti-CD68 (red) antibodies and DAPI (blue). Representative images are shown.

Figure 3: Study Design



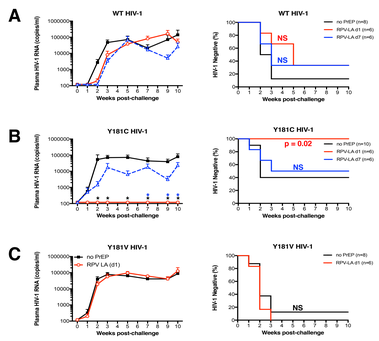
(A) Pharmacokinetics were determined in plasma and female genital tract tissues of 26 mice for either RPV LA or tenofovir (TNV) and emtricitibine (FTC). (B) 68 mice were used for PrEP treatment or controls and then vaginal challenge with WT, Y181C, or Y181V HIV-1. Plasma viremia was measured for up to 10 weeks using the HIV-1 single copy assay (SCA; Palmer et al. J Clin Microbiol 2003). Plasma HIV-1 RNA from the last time point of mice with breakthrough infection were analyzed by single-genome sequencing (SGS; Palmer et al. J Clin Microbiol 2005).

Figure 4: Plasma & Genital RPV Concentrations in Mice



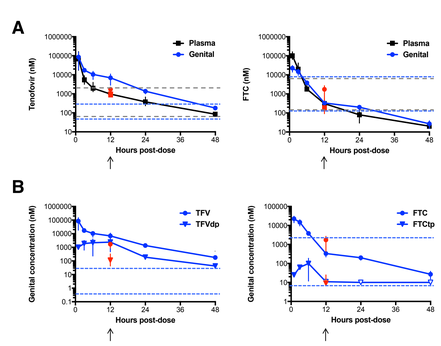
A single 150 mg/kg dose of RPV LA was administered IM to 14 mice. RPV concentrations were measured in plasma and female genital tract at 1, 3, 6, 12, 24, and 48 hours and 7 days post-dose. The dashed lines represent the range of measured concentrations from plasma (grey) or genital tract tissue (blue) from women over 28 days post-dose (Jackson et al. Clin Pharmacol Ther 2014). The red line indicates the protein-adjusted EC90 (12 ng/ml or 33 nM; Mora-Peris et al. J Antimicrob Chemother 2014).

Figure 5: Effect of RPV LA PrEP on HIV-1 Vaginal Transmission



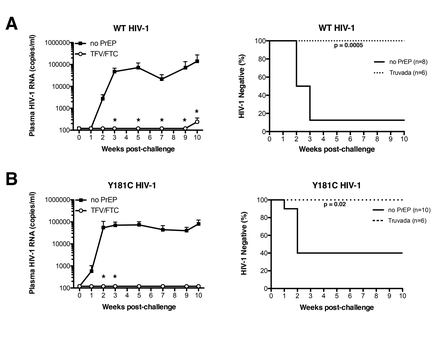
Average plasma HIV-1 RNA levels (left) and Kaplan-Meier curves (right) are shown in animals challenged with (A) WT, (B) Y181C, or (C) Y181V HIV-1 after no treatment or at days 1 or 7 after RPV LA dose. p values < 0.05 are shown for time points comparing untreated vs. RPV LA d1 groups (*) or untreated vs. RPV LA d7 groups (#). p values are shown between untreated and RPV LA treatment groups. NS denotes a p value > 0.05 (not significant).

Figure 6: Plasma & Genital TNV/FTC Concentrations in Mice



A single dose of TFV (150 mg/kg) and FTC (100 mg/kg) was administered SC to 12 mice. (A) TFV and FTC concentrations were measured in plasma (black) and female genital tract (blue). The dashed lines represent the range of measured TFV and FTC concentrations from human plasma (grey) or genital tract tissues (blue). (B) TFV and FTC (blue circles) and TFVdp and FTCtp (blue triangles) were measured in the genital tract. The blue dashed lines represent the range of measured TFVdp and FTCtp concentrations from human tissues. Two doses of TFV and FTC (150 and 275 mg/kg) were administered 24 hours apart to 3 animals and the drugs and metabolites were measured 12 hours after the last dose (red symbols).

Figure 7: Effect of TNV/FTC PrEP on Vaginal HIV-1 Transmission



Average plasma HIV-1 RNA levels (left) and Kaplan-Meier curves (right) in animals challenged with (A) WT or (B) Y181C HIV-1 after no treatment or during 5 days of TFV/FTC treatment. Error bars represent the standard errors of the mean. The limit of quantitation was 120 HIV-1 RNA copies/ml. p values < 0.05 are shown for time points comparing untreated vs. TFV/FTC groups (*). p values are shown between treatment groups.

Table 1: New RT Mutations Detected in Plasma HIV-1 by SGS

Group	Percentage of Mice with New RPV-Resistant Mutations	Percentage of Mice with New RPV-Resistant Mutations > 5%	Percentage of Mice with M184I
WT Untreated	0 (0/1)	0 (0/1)	0 (0/1)
Y181C Untreated	0 (0/2)	0 (0/2)	0 (0/2)
Y181V Untreated	50 (1/2)	50 (1/2)	0 (0/2)
All Untreated	**20 (1/5)**	**20 (1/5)**	**0 (0/5)**
WT RPV (d1)	25 (1/4)	25 (1/4)	0 (0/4)
WT RPV (d7)	33 (1/3)	0 (0/3)	0 (0/3)
Y181C RPV (d7)	0 (0/3)	0 (0/3)	33 (1/3)
Y181V RPV (d1)	33 (2/6)	0 (0/6)	0 (0/6)
All RPV-Treated	**25 (4/16)[a]**	**6 (1/16)[a]**	**6 (1/16)[a]**

[a] Frequencies of total treated group were not significantly different than frequencies of the untreated group ($p > 0.05$)

Table 2: NNRTI-Associated Mutations Detected in Plasma by SGS

Challenge Virus	Animal	Treatment (Day of Challenge)	NNRTI-Associated Mutations (Frequency)
WT	448-004	None	None (0/42)[a]
	255-002	RPV LA (d1)	None (0/43)
	255-007		None (0/44)
	255-022		H221Y (1/47)
	255-044		**K101E/E138K (1/47), E138K (3/47),** V179I (1/47)
	912-008	RPV LA (d7)	None (0/40)[a]
	912-014		None (0/44)[a]
	943-004		**E138Q (1/46)**[a]
	255-042		ND
Y181C	448-024	None	C181Y (1/46)[a,b], H221Y (1/46)
	912-007		None (0/42)[a]
	912-009	RPV LA (d7)	None (0/44)[a]
	912-017		C181Y (1/46)[a,b], M184I (2/46)
	943-003		None (0/46)[a]
Y181V	255-031	None	**E138K (4/42)**
	943-021		None (0/47)
	255-006	RPV LA (d1)	Y188H (1/45)
	255-013		V179I (2/43)
	255-025		V179I (3/46)
	255-038		**E138K (1/44),** P225H (1/44)
	255-045		**E138K (2/44),** V179I (1/44)
	255-046		None (0/46)

[a] No mutations detected by population sequencing of this sample
[b] Reversion to WT, not linked to additional resistance mutations
ND, not done
Bold purple, RPV-resistant mutations

Conclusions

- **RPV LA plasma and genital tract concentrations in female BLT mice were similar to those detected in human women.**
- **Biologically relevant RPV concentrations (day 7 post-dose) only slightly delayed WT or Y181C HIV-1 dissemination.**
- **High RPV concentrations (day 1 post-dose) inhibited transmission of Y181C HIV-1, but not Y181V HIV-1.**
- **Daily TNV/FTC treatment 2 days before and 3 days after WT or Y181C HIV-1 challenge inhibited transmission.**
- **RPV LA did not lead to detectable NNRTI resistance with SGS after 10 weeks post-challenge in the majority of animals infected during RPV LA PrEP.**
- **RPV and FTC resistance mutations (E138K, M184I) arose in both the untreated and the treated animals at similar rates, the majority of which have been shown to be APOBEC3-mediated.**
  - **The impact of low frequency mutations, particularly with low-level resistance, on subsequent ART requires further investigation.**

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