874

Distinct Pharmacodynamic Activity of Rilpivirine in Mucosal Explant Tissue

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Introduction

- Rilpivirine (RPV) is a potent, second generation nonnucleoside reverse transcriptase inhibitor.
- A long-acting (LA) formulation has been developed for treatment and is being considered for HIV prevention.
- Two dose escalation clinical trials have been completed in men and women.
  - SSAT040 evaluated 300 mg, 600 mg, and 1200 mg of RPV LA; men were dosed with 600 mg only.
  - MWRI-01 evaluated 600 mg and 1200 mg of RPV LA and incorporated the *ex vivo* challenge assay.
- Drug penetration into rectal tissue (93 and 78 ng/ml) was approximately twice as much as compared to vaginal tissue (38 and 39 ng/ml) in both studies for the 600 mg dose.
- Significant suppression of HIV infection was noted 1 month post-injection for rectal tissue, but not for cervical or vaginal tissue (*ex vivo* challenge assay) in participants receiving the 600 and 1200 mg doses in MWRI-01.
- Our interest was to define the concentration of RPV (pharmacokinetics [PK]) needed to prevent HIV infection (pharmacodynamics [PD]) in mucosal tissue.

Methods

- RPV was supplied by Janssen Pharmaceutica, Belgium.
- HIV-1_{BaL} was used for the experiments here and is the same virus used for the *ex vivo* challenge assay in MWRI-01. The 50% tissue culture infectious dose ($TCID_{50}$) was determined in peripheral blood mononuclear cells.
- The *in vitro* 90% effective concentration (EC_{90}) and 90% cytotoxic concentration (CC_{90}) of RPV for HIV-1_{BaL} was determined by the 4-parameter Emax model [y = min + (maxmin)/(1 + (x/EC_{90})^(-Hillslope))] (SigmaPlot11, Systat Software, Inc., San Jose, CA) using TZM-bl assay data.
- For drug efficacy, 10-fold dilutions of RPV were applied to the basolateral chamber of polarized tissue explants for 24 h followed by infection with HIV-1_{BaL}(5×10^4 $TCID_{50}$ for ectocervical tissue and 10^4 $TCID_{50}$ colonic tissue) added to the apical surface for an additional 24 h. The explants were washed and supernatant was collected and replenished every 3-4 days for 21 days. HIV infection was measured in the supernatants by HIV p24 ELISA.
- In some experiments, explants were set-up in quadruplicate and the second set of explants were collected after 48 h of culture for drug quantification using a validated LC-MS/MS method. Rilpivirine concentrations below the level of quantification (BLQ) were imputed to half the lower limit of quantification (LOQ).
- Correlations between log_{10} transformed p24 levels on day 21 and log_{10} transformed drug levels were defined by GraphPad Prism® (V5.02) software (La Jolla, CA) using a linear, least-squared regression where the probability value of the slope indicated a relationship that was significantly different to the zero slope ($P < 0.05$). To define the tissue effective dose, the % inhibition was calculated for each explant based on setting the untreated control day 21 p24 as 100% infected. Using the 4-parameter Emax model, the EC_{90} was calculated.

Acknowledgement

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Results

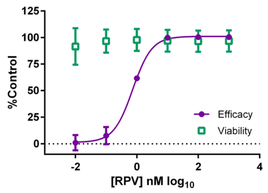
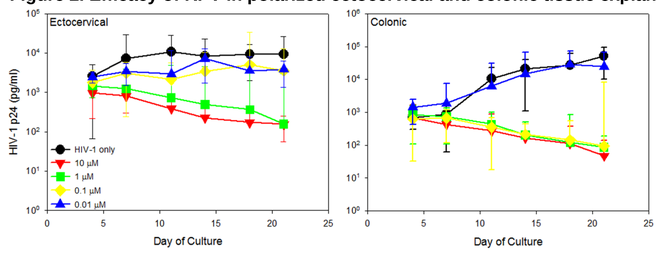


Figure 1. *In vitro* EC_{90} and CC_{90} of RPV. The EC_{90} was 1.67 nM and the CC_{90} was 5827 nM. RPV is a potent, non-toxic drug.

Figure 2. Efficacy of RPV in polarized ectocervical and colonic tissue explants.



- Viral replication was suppressed in the ectocervical tissue explants dosed with 10 µM of RPV and in colonic tissue explants dosed with 1 µM of RPV as compared to HIV-1 only controls (**Figure 2; Table 1**).
- Lower concentrations of RPV added to the basolateral medium showed partial inhibition with loss at 0.01 µM for both tissue types (**Figure 2; Table 1**).

Table 1. Inhibition of HIV in ectocervical and colonic tissue explants treated with basolateral RPV.

[RPV][a]	Ectocervical explants		Colonic explants	
	# protected / # tested	% inhibition[b]	# protected / # tested	% inhibition[b]
10 µM	10 / 10	98%	9 / 9	99.8%
1 µM	7 / 12	98%	15 / 15	99.8%
0.1 µM	2 / 10	62%	11 / 15	99.7%
0.01 µM	0 / 6	59%	2 / 8	31.0%

[a]Concentration of RPV added to the basolateral supernatant.
[b]% inhibition was calculated using averaged median p24 values from day 21 of culture shown in Fig 2.

Table 2. RPV concentration in ectocervical and colonic tissue explants.

[RPV] added to culture	Ectocervical explants		Colonic explants	
	[RPV] (mean ± SD)	%RPV in tissue[a]	[RPV] (mean ± SD)	%RPV in tissue[a]
10 µM	282 ± 116 ng/mL	8%	4624 ± 1745 ng/mL	126%
1 µM	20 ± 11 ng/mL	6%	467 ± 103 ng/mL	128%
0.1 µM	BLQ	—	47 ± 10 ng/mL	127%
0.01 µM	BLQ	—	3.4 ± 1.2 ng/mL	100%

[RPV], Rilpivirine concentration; BLQ, below limit of quantification
[a]Relative tissue concentration as a percentage of the total amount of RPV added to the culture.

- <10% of the RPV was quantified in the ectocervical tissue.
- RPV in colonic tissue appeared to be in equilibrium with the culture medium.
- Colonic tissue contained 16- to 23-fold more RPV than ectocervical tissue.
- Better penetration of RPV into colonic than ectocervical tissue.

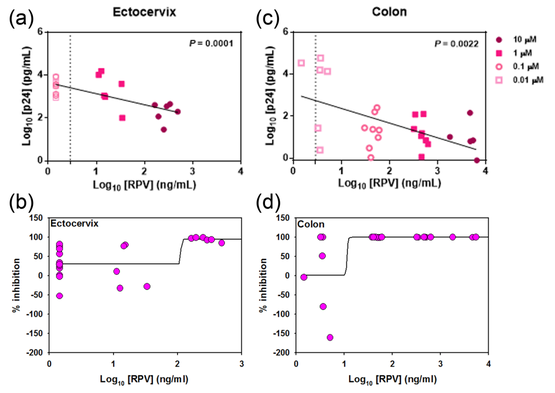


Figure 3. PK and PD activity of RPV modeled in mucosal tissue.

- Data BLQ were imputed as ½ LOQ (0.6 log_{10} ng/mL) and indicated with a vertical dotted line.
- RPV tissue concentrations (log_{10} ng/mL) were plotted against day 21 p24 log_{10} pg/mL with significance noted for ectocervical **(a)** and colonic **(c)** explants.
- Dose response relationship between [RPV] and % inhibition defined the EC_{90} for ectocervical (99 ng/mL; 271 nM) **(b)** and colonic (16.33 ng/mL 45 nM) **(d)** explants.

Conclusions

- RPV was effective *in vitro* against HIV-1_{BaL} as measured in TZM-bl and mucosal tissue assays.
- RPV penetrated colonic tissue better than ectocervical tissue by >10-fold *in vitro*, which was consistent with the PK data from the two clinical trials.
- SSAT040 (600 mg) and MWRI-01 (600 mg) studies demonstrated similar concentrations of RPV in rectal (93 and 78 ng/ml) and vaginal (39 and 38 ng/ml) tissues.
- Importantly, the data presented here suggest that the concentrations of RPV in the rectal tissue exceeded by ~5-fold what would be needed to suppress viral infection (>16 ng/ml), but was 2.5-fold below the suppressive levels needed in cervical / vaginal tissue (>99 ng/ml).
- This was confirmed by the *ex vivo* challenge data from MWRI-01; HIV infection was suppressed in rectal tissue but not in cervical / vaginal tissues.
- Our data suggest that after parenteral dosing, sufficient levels of RPV appear to be present in the colon, but higher concentrations may be needed in the cervix / vagina for protection against HIV acquisition.

References

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