Distinct Pharmacodynamic Activity of Rilpivirine in Mucosal Explant Tissue

Charlene S. Dezzutti, PhD1,2, Laura J. Else, PhD3, Sarah E. Yandura2, Cory Shetler2, Julie Russo2, David J. Back, PhD3, and Ian McGowan, MD, PhD1,2
1School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA, 2Magee-Womens Research Institute, Pittsburgh, PA, USA, 3Department of Molecular & Clinical Pharmacology, University of Liverpool, Liverpool, UK

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.

• MWR1-01 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 MWR1-01.

• 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-1a was used for the experiments here and is the same virus used for the ex vivo challenge assay in MWR1-01. The 50% tissue culture infectious dose (TCID50) was determined in peripheral blood mononuclear cells.

• The in vitro 90% effective concentration (EC90) and 90% cytotoxic concentration (CC90) of RPV for HIV-1a was determined by the 4-parameter Emax model [y = min + (maximin)/1 + (EC50-x)•Hillshape] (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-1a (5×10^5 TCID50 for ectocervical tissue and 10^5 TCID50 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 log10-transformed p24 levels on day 21 and log10-transformed drug levels were defined by GraphPad Prism® (V5.02) software. Rilpivirine concentrations below the level of quantification (BLQ) were imputed to half the lower limit of quantification (LOQ).

• 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).

• Our data suggest that after parenteral dosing, sufficient levels of RPV appear to be present in the rectal tissue but not in cervical / vaginal tissues. Better penetration of RPV into colonic than ectocervical tissue.

Conclusions

• RPV was effective in vitro against HIV-1a as measured in TZM-bl and mucosal tissue assays.

• RPV penetration in colonic tissue was better than ectocervical tissue by >10-fold in vitro, which was consistent with the PK data from the two clinical trials.

• 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 MWR1-01.

• 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 MWR1-01.

• SSAT040 (600 mg) and MWR1-01 (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).

• 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.

Figure 1. In vitro EC50 and CC50 of RPV. The EC50 was 1.67 nM and the CC50 was 8527 nM. RPV is a potent, non-toxic drug.

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

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

<table>
<thead>
<tr>
<th>RPV concentration (µM)</th>
<th>Ectocervical explants</th>
<th>Colonic explants</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.001 µM</td>
<td>10/10 (100%)</td>
<td>9/0 (90%)</td>
</tr>
<tr>
<td>1 µM</td>
<td>7/12 (60%)</td>
<td>15/15 (100%)</td>
</tr>
<tr>
<td>10 µM</td>
<td>0/0 (0%)</td>
<td>0/0 (0%)</td>
</tr>
</tbody>
</table>

*Inhibition was calculated using averaged median p24 values from day 21 of culture shown in Fig 2

Acknowledgement

This work was supported by a grant from the Bill and Melinda Gates Foundation, #OPP1043535.