AIDS RESEARCH AND HUMAN RETROVIRUSES
Volume 37, Number 3, 2021
© Mary Ann Liebert, Inc.
DOI: 10.1089/aid.2020.0155

Metabolism of Long-Acting Rilpivirine After Intramuscular Injection: HIV Prevention Trials Network Study 076 (HPTN 076)

Herana Kamal Seneviratne,[1,*] Joseph Tillotson,[1,*] Julie M. Lade,[2] Linda-Gail Bekker,[3] Sue Li,[4] Subash Pathak,[4] Jessica Justman,[5] Nyaradzo Mgodi,[6] Shobha Swaminathan,[7] Nirupama Sista,[8] Jennifer Farrior,[8] Paul Richardson,[9] Craig W. Hendrix,[1] and Namandje N. Bumpus[1,2]

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.

Abstract

A long-acting injectable formulation of rilpivirine (RPV), a non-nucleoside reverse transcriptase inhibitor, is currently under investigation for use in human immunodeficiency virus (HIV) maintenance therapy. We previously characterized RPV metabolism after oral dosing and identified seven metabolites: four metabolites resulting from mono- or dioxygenation of the 2,6-dimethylphenyl ring itself or either of the two methyl groups located on that ring, one N-linked RPV glucuronide conjugate, and two O-linked RPV glucuronides produced via glucuronidation of mono- and dihydroxymethyl metabolites. However, as is true for most drugs, the metabolism of RPV after injection has yet to be reported. The phase II clinical trial HPTN 076 enrolled 136 HIV-uninfected women and investigated the safety and acceptability of long-acting injectable RPV for use in HIV pre-exposure prophylaxis. Through the analysis of plasma samples from 80 of these participants in the active product arm of the study, we were able to detect 2 metabolites after intramuscular injection of long-acting RPV, 2-hydroxymethyl-RPV, and RPV N-glucuronide. Of the total of 80 individuals, 72 participants exhibited detectable levels of 2-hydroxymethyl-RPV in plasma samples whereas RPV N-glucuronide was detectable in plasma samples of 78 participants. In addition, RPV N-glucuronide was detectable in rectal fluid, cervicovaginal fluid, and vaginal tissue. To investigate potential genetic variation in genes encoding enzymes relevant to RPV metabolism, we isolated genomic DNA and performed next-generation sequencing of *CYP3A4*, *CYP3A5*, *UGT1A1* and *UGT1A4*. From these analyses, four missense variants were detected for *CYP3A4* whereas one missense variant and one frameshift variant were detected for *CYP3A5*. A total of eight missense variants of *UGT1A4* were detected, whereas two variants were detected for *UGT1A1*; however, these variants did not appear to account for the observed interindividual variability in metabolite levels. These findings provide insight into the metabolism of long-acting RPV and contribute to an overall understanding of metabolism after oral dosing versus injection.
ClinicalTrials.gov Identifier: NCT02165202

Keywords: Rilpivirine, long-acting, intramuscular injection, HIV prevention, metabolism

[1]Division of Clinical Pharmacology, Department of Medicine, the Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.
[2]Department of Pharmacology and Molecular Sciences, the Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.
[3]The Desmond Tutu HIV Centre, University of Cape Town, Cape Town, South Africa.
[4]Statistical Center for HIV/AIDS Research & Prevention (SCHARP), Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.
[5]ICAP at Columbia, Mailman School of Public Health, and Division of Infectious Diseases, Vagelos College of Physicians and Surgeons, Columbia University, New York, New York, USA.
[6]University of Zimbabwe–University of California, San Francisco (UZ-UCSF) Collaborative Research Programme, Harare, Zimbabwe.
[7]Department of Medicine, Rutgers New Jersey Medical School, Newark, New Jersey, USA.
[8]FHI 360, Durham, North Carolina, USA.
[9]Department of Pathology, the Johns Hopkins University School of Medicine, Baltimore, Maryland, USA.
*These two authors contributed equally to this work.

Introduction

Rilpivirine (RPV) is a second-generation non-nucleoside reverse transcriptase inhibitor (NNRTI) of human immunodeficiency virus (HIV).[1,2] As a diarylpyrimidine, RPV has an inherent degree of flexibility and rotational freedom that enables it to bind in the NNRTI binding pocket of the HIV reverse transcriptase enzyme in several conformations, thereby allowing for adaptation to potential resistance mutations.[3] RPV was FDA-approved in 2011 for administration to treatment-naive HIV-infected adults; because it was found to be highly efficacious and well tolerated with chronic oral administration, it was concurrently developed into a long-acting injectable formulation.[4] A long-acting formulation of RPV may improve adherence since it requires less frequent dosing as compared with the current daily dosing strategy of RPV oral formulations. In addition to potential use in HIV maintenance therapy, long-acting RPV was being considered for use in HIV prevention as pre-exposure prophylaxis (PrEP).[5]

After oral administration, drugs are absorbed through the gastrointestinal tract where they reach the liver and undergo first-pass hepatic metabolism. The liver contains a high abundance of drug-metabolizing enzymes, including the cytochromes P450 (CYP; P450) and UDP-glucuronosyltransferases (UGT).[6] Drug metabolism facilitates the clearance of administered drugs from the body. The most common enzymes involved in the first-pass metabolism, P450s, convert drugs into more polar products to facilitate their elimination from the body.[7] In phase II metabolism, drugs and/or products of first-pass metabolism are conjugated with a hydrophilic endogenous compound. Glucuronidation represents the major pathway in phase II metabolism, and glucuronidated products are eliminated from the body through urine or feces.[8] In contrast, drugs administered via intramuscular injections largely bypass first-pass hepatic metabolism. Although extrahepatic organs may be involved in the clearance of drugs delivered via alternative routes such as intramuscular injections, the pathways of their metabolism are largely unexplored. In addition, little is known about the differences in the metabolism of drugs administered via oral routes versus intramuscular injections. Moreover, the pathways of drug metabolism after intramuscular injections may yield insights into their efficacy and safety profiles.

Previously, we characterized RPV metabolism after oral dosing and identified seven metabolites: four mono- or dihydroxylated metabolites, one N-linked RPV glucuronide conjugate, and two O-linked RPV glucuronides produced via glucuronidation of mono- and dihydroxymethyl metabolites.[9] In the same study, we elucidated the *in vitro* routes of RPV metabolism by using human liver microsomes and cDNA-expressed enzyme assays. These analyses indicated that CYP3A4 and CYP3A5 are primarily responsible for the oxidative metabolism of RPV. In addition, it was found that RPV and a monomethylhydroxylated metabolite of RPV are primarily metabolized via glucuronidation by UGT1A4 and UGT1A1, respectively.[9] However, a role for these enzymes in metabolizing a drug delivered via an injection is undefined and the genes encoding enzymes CYP3A5, UGT1A1, and UGT1A4 are polymorphic.

In this work, we investigated the metabolism of orally administered RPV as well as long-acting RPV delivered via intramuscular injections in HIV Prevention Trials Network 076 (HPTN 076) study participants. The HPTN 076 study was a multi-site, double-blinded, two-arm (2:1), randomized, phase II clinical trial conducted to investigate the safety and acceptability of a long-acting injectable (1,200 mg dosed six times at 8 week intervals) for use in HIV PrEP.[10] HIV-uninfected women ($n = 136$) were recruited across four cities for this study: Bronx, New York; Newark, New Jersey; Cape Town, South Africa; and Harare, Zimbabwe. Initial findings from this study have demonstrated the high acceptability of long-acting RPV for long-acting injectable PrEP delivery over both oral and vaginal methods among study participants (U.S. and African women).[10] In addition, the safety and tolerability of long-acting RPV has been reported recently.[11] The goals of this work were to characterize RPV metabolites *in vivo* by using plasma, rectal fluid, cervicovaginal fluid, and vaginal tissue samples obtained from HPTN 076 study participants, and to investigate the presence of variants of *CYP3A4*, *CYP3A5*, *UGT1A1*, and *UGT1A4* by using next-generation targeted sequencing. After the injection phase, two metabolites, 2-hydroxymethyl RPV and RPV N-glucuronide were detected in plasma samples of the participants. In addition, RPV N-glucuronide was detectable in rectal fluid, cervicovaginal fluid, and vaginal tissue. From next-generation targeted sequencing analyses, four missense variants were detected for *CYP3A4* whereas *UGT1A4* exhibited eight missense variants. In sum, results from this study yield novel insights into the metabolism of long-acting RPV.

Materials and Methods

Chemicals and reagents

RPV was provided through the National Institutes of Health AIDS Reagents Program. 2-Hydroxymethyl RPV and rilpivirine-d_6 (RPV-d_6) were obtained from Toronto Research Chemicals (Toronto, ON, Canada). All solvents used were high-performance liquid chromatography (HPLC) grade and obtained from Fisher Scientific (Hampton, NH), unless otherwise specified.

Clinical samples

The HPTN 076 study was carried out as reported by Tolley *et al.*[10] The study protocol was approved by the institutional review board or ethics committee at each research site. All research participants provided voluntary written informed consent to participate in the HPTN 076 study. Whole blood and plasma were obtained from HIV-uninfected females ($n = 136$) enrolled in the HPTN 076 trial across four study sites: Bronx Prevention Center CRS, Bronx, NY, USA ($n = 19$); New Jersey Medical School Clinical Research Center CRS, Newark, NJ, USA ($n = 17$); Emavundleni CRS, Cape Town, South Africa ($n = 48$); and Spilhaus Clinical Research Site, Harare, Zimbabwe ($n = 52$). The median age of the research participants was 31 years and overall, 94% (128/136 individuals) of them were Black/African American. The details of HPTN 076 research participants have been published previously.[10] Baseline demographics of the study population that was used for RPV metabolism study are shown in Table 1. A lead-in period with oral RPV was conducted to screen for initial safety and tolerability of RPV. After daily oral administration of RPV, plasma samples used for metabolite analysis were obtained at week 4 whereas post-injection plasma samples for metabolite analysis were collected just before the fifth

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.

Table 1. Summary of Baseline Demographics of the Research Participants Included in the Rilpivirine Metabolism Study

	RPV (N = *83*)
Site	
U.S.	23/83 (28%)
Africa	60/83 (72%)
Age	
Mean (IQR)	31 (27–36)
Median	31
U.S. race (*N* = 23)	
Asian	1/23 (4.3%)
Black/African American	18/23 (78.3%)
White	3/23 (8.3%)
Other	1/23 (4.3%)
Zimbabwe race (*N* = 35)	
Shona	33/35 (94.3%)
Ndebele	1/35 (2.9%)
Other African Group	1/35 (2.9%)
Cape Town race (*N* = 25)	
Black	25/25 (100%)
Weight, kg	
Mean (IQR)	77 (67–87)
Median	75
BMI	
Mean (IQR)	31 (26–35)
Median	30

IQR, interquartile range; RPV, rilpivirine.

injection (week 36). In this work, we analyzed 83 post-oral plasma samples whereas only 80 post-injection samples were available. Cervicovaginal fluid, rectal fluid, and vaginal tissue samples analyzed for metabolites were collected just before the fifth or sixth injection (week 36 or 44, respectively). Vaginal tissue was collected only from participants in the United States. (Bronx/Newark, USA). Only 79 rectal fluid samples were obtained for this analysis.

Measurement of RPV metabolites

Plasma, cervicovaginal fluid, rectal fluid, and vaginal tissue samples were analyzed for the presence of RPV metabolites by using an ultra-HPLC-tandem mass spectrometry assay as previously published.[9] Briefly, analytes of interest from plasma, cervicovaginal fluid, and rectal fluid aliquots were extracted by adding ice-cold acetonitrile containing the internal standard (IS), RPV-d6 at 100 ng/mL (200 μL for plasma and 100 μL for cervicovaginal fluid and rectal fluid). Vaginal tissue was thawed and extracted by adding 100 μL of 70% methanol supplemented with 100 ng/mL RPV-d6 for every 5 mg of tissue and homogenized. Samples were vortexed, incubated at room temperature for 10 min, and centrifuged for 10 min at 10,000 *g* at 4°C. Supernatant was collected and dried under vacuum. Samples were reconstituted in methanol (200 μL for plasma, 100 μL for cervicovaginal fluid and rectal fluid, and 50 μL for tissue), vortexed, and incubated at room temperature for 10 min before centrifugation for 5 min at 10,000 *g* at 4°C. Resulting supernatants were collected for mass spectral analyses, injecting 10 μL per sample from plasma, cervicovaginal fluid, and rectal fluid and 5 μL per sample from tissue. Liquid chromatography-tandem mass spectrometry analyses were carried out by using a Dionex Ultimate 3000 uHPLC system coupled to a TSQ Vantage Triple Stage Quadrupole mass spectrometer (Thermo Scientific).

In the selected reaction monitoring mode, fragment ions were detected by positive ionization using the following transitions (Q1 → Q3): 383 → 222 *m/z* (monohydroxylated RPV), 399 → 183 *m/z* (dihydroxylated RPV), 399 → 196 *m/z* (dihydroxylated RPV), 543 → 367 *m/z* (RPV glucuronide conjugate), 559 → 383 *m/z* (monohydroxylated RPV glucuronide conjugate), and 575 → 399 *m/z* (dihydroxylated RPV glucuronide conjugate). The limit of quantitation for 2-hydroxymethyl-RPV in plasma samples was 0.781 ng/mL. Peaks detected that fell below the limit of quantitation are reported as detectable. The range for the quantitation of plasma 2-hydroxymethyl-RPV was 0.781–1,600 ng/mL. The standard curves were calculated by using GraphPad Prism (San Diego, CA) by taking the ratio of analyte peak area over IS and fit by using a weighted ($1/y^2$) linear regression, and concentrations of samples were interpolated by using the curves.

Statistical analysis

Statistical analyses were performed by using GraphPad Prism (San Diego, CA). Kruskal–Wallis tests followed by Dunn's test were performed, and significance was denoted as follows: $*p \le .05$; $**p \le .01$; $***p \le .001$. The confidence interval used was 95%.

Genomic DNA isolation and sample preparation for next-generation sequencing

Genomic DNA was isolated from 200 μL of whole blood by using the QIAamp 96 DNA Blood Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Purified DNA was eluted by using 200 μL of elution buffer.

Samples were prepared following the TruSeq custom amplicon library preparation kit guide (Illumina, San Diego, CA) by using 250 ng of template DNA per reaction. Agencourt AMPure XP beads (Beckman Coulter, Inc., Brea, CA) were used for PCR clean-up. The final pooled DNA library (6 μL) was diluted in 594 μL HT1 buffer and spiked with 1% PhiX. One technical control was included per sample batch, and runs were sequenced by using an Illumina MiSeq sequencing platform generating 150 base pair reads.

Next-generation sequencing targeted enrichment design

Sequencing was performed by using the Illumina TruSeq custom amplicon v1.5 kit (San Diego, CA). Custom probes targeting the exonic regions of *CYP3A4*, *CYP3A5*, *UGT1A1*, and *UGT1A4* were generated *in silico* by using Illumina DesignStudio software. The chromosomal coordinates used were as follows: *CYP3A4* 7:99354583–7:99381811; *CYP3A5* 7:99245813–7:99277621; *UGT1A1* 2:234668919–2:234681945; *UGT1A4* 2:234627438–2:234681945. The final design included 120 amplicons.

Next-generation sequencing data analysis

Secondary analysis of the base calls and Phred-like quality score (Qscore) generated by Real Time Analysis software was performed by using on-instrument MiSeq Reporter software.

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.

Reads were mapped to the GRCh37 (hg19) reference assembly by using a banded Smith-Waterman algorithm, and variant calling was carried out by using the Genome Analysis Toolkit. Variant call format files were annotated by using Illumina VariantStudio software. Raw variant calls were filtered by applying a read depth threshold >1,500 bases per variant, a minimum base call Qscore of 30 (error rate of 1 in 1,000), and an alternate variant frequency >45%, followed by visual inspection using the Integrative Genome Viewer. Variants were ultimately cross-referenced with the National Center for Biotechnology Information database of Single Nucleotide Polymorphisms, and variant alleles were assigned by using the Karolinska Institute's Human Cytochrome P450 Allele Nomenclature Database and PharmGKB.[12]

Results

Detection of RPV metabolites in plasma samples of HPTN 076 participants following oral administration of RPV versus long-acting RPV delivery via an intramuscular injection

Baseline demographics of the study population that was used for this RPV metabolism study are shown in Table 1. To probe the metabolism of RPV after oral administration versus delivery via a long-acting injectable, we measured RPV metabolites in HPTN 076 participants after both oral dosing and intramuscular injection. Of the total of 136 study participants, 83 received 25 mg of RPV once daily for 4 weeks. After this oral lead-in phase, plasma samples were obtained from research participants ($n = 83$) at week 4. From these analyses, of the four established oxidative metabolites of RPV, only 2-hydroxymethyl-RPV was detectable in any of the participants after the oral phase. Specifically, 2-hydroxymethyl-RPV was detected in plasma samples of 75 study participants (90%). Of these, 58 participants (70% of 83) exhibited quantifiable levels of 2-hydroxymethyl-RPV in their plasma samples (Bronx/Newark, USA $n = 9$, Cape Town, South Africa $n = 16$, Harare, Zimbabwe $n = 33$) (Fig. 1A). The mean 2-hydroxymethyl-RPV plasma level was 3.04 ± 1.60 ng/mL, and the distribution of metabolite plasma levels across study sites is shown in Figure 1A. In addition to 2-hydroxymethyl-RPV, we were able to readily detect another metabolite, RPV N-glucuronide in 78 of the 83 (94%) plasma samples analyzed (Bronx/Newark, USA $n = 20$, Cape Town, South Africa $n = 23$, Harare, Zimbabwe $n = 35$) (Fig. 1B). We were not able to quantitate the levels of

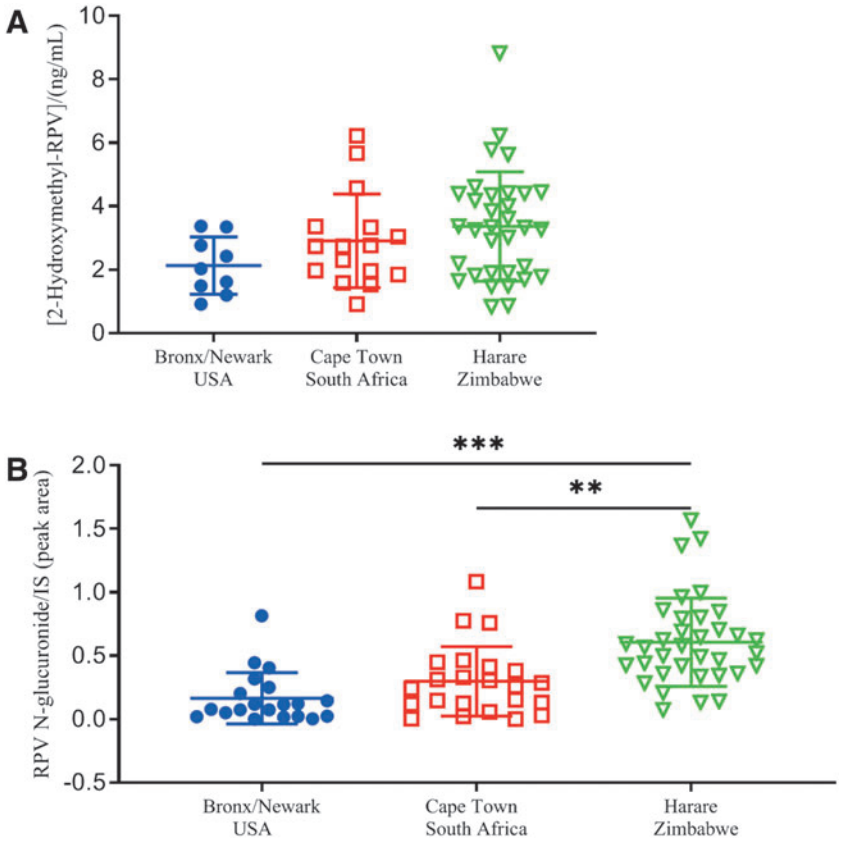

FIG. 1. Detection of 2-hydroxymethyl-RPV and RPV N-glucuronide in plasma samples of HTPN 076 research participants after oral dosing of RPV (25 mg, once-daily) for 4 weeks. **(A)** 2-hydroxymethyl-RPV and **(B)** RPV N-glucuronide in plasma samples of HPTN 076 study participants were detected by using an ultra-high-performance liquid chromatography-tandem mass spectrometry assay, as previously published.[9] The 2-hydroxymethyl-RPV metabolite was quantified by using a synthetic standard, and the levels of 2-hydroxymethyl-RPV are represented as ng/mL. Due to the lack of a synthetic standard for RPV N-glucuronide, data are represented as a peak area ratio to the IS, RPV-d6. A total of 83 plasma samples collected from study sites, Bronx/Newark, USA $n = 23$, Cape Town, South Africa $n = 25$, Harare, Zimbabwe $n = 35$ were analyzed. Statistical significance was denoted as follows: $^{**}p \leq .01$; $^{***}p \leq .001$. IS, internal standard; RPV, rilpivirine.

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.

RPV N-glucuronide due to the absence of a synthetic standard (there were several failed synthesis attempts); therefore, we utilized the peak area ratio of RPV N-glucuronide to RPV-d_6 (as an IS) to qualitatively compare across participants.

We next analyzed plasma samples of HPTN 076 study participants after intramuscular injection at week 36 (8 weeks after the fourth injection) ($n=80$). We took an unbiased approach and we looked for metabolites by using a non-targeted mass spectrometry method that records full scan spectra of analytes. We then employed a targeted mass spectrometry assay to specifically detect the seven known RPV metabolites. Of the 80 plasma samples analyzed, 72 participants (90%) had detectable levels of 2-hydroxymethyl-RPV in plasma. However, only 21 research participants (26% of 80) exhibited quantifiable levels of 2-hydroxymethyl-RPV in plasma during the injection phase (Bronx/Newark, USA $n=7$, Cape Town, South Africa $n=9$, Harare, Zimbabwe $n=5$) (Fig. 2A). The mean 2-hydroxymethyl-RPV plasma level was 1.10 ± 0.37 ng/mL (Fig. 2A). As observed in the oral phase, we were able to detect RPV N-glucuronide after intramuscular injection. Of the 80 plasma samples analyzed, 78 (98%) exhibited detectable levels of RPV N-glucuronide (Bronx/Newark, USA $n=22$, Cape Town, South Africa $n=22$, Harare, Zimbabwe $n=34$) (Fig. 2B). In addition, the variation of the plasma levels of both RPV metabolites, 2-hydroxymethyl-RPV and RPV N-glucuronide in participants after oral dosing versus injection is shown in Figure 3.

Detection of RPV metabolites in rectal fluid, cervicovaginal fluid, and vaginal tissue samples of HPTN 076 participants following long-acting RPV delivery via an intramuscular injection

In addition to plasma samples, we obtained rectal fluid, cervicovaginal fluid, and vaginal tissue samples from HPTN 076 study participants after the injection phase at weeks 36 or 44. Of the 79 rectal fluid samples obtained and analyzed, 30 participants (38%) had 2-hydroxymethyl-RPV in their rectal fluids with a mean value of 0.060 ± 0.17 ng/mg of sample (Bronx/Newark, USA $n=8$, Cape Town, South Africa $n=13$, Harare, Zimbabwe $n=9$) (Fig. 4A). In contrast, we were not able to detect the RPV metabolite, 2-hydroxymethyl-RPV in either cervicovaginal fluid or vaginal tissue samples. However, we were able to detect the RPV N-glucuronide in rectal fluid, cervicovaginal fluid, and vaginal tissue (Figs. 4B–D). Of the 79 rectal fluid analyzed, 33 participants (42%) exhibited detectable levels of RPV N-glucuronide (Bronx/Newark, USA $n=10$, Cape Town, South Africa $n=11$, Harare, Zimbabwe $n=12$) (Fig. 4B). Eighty cervicovaginal fluid samples were analyzed for RPV N-glucuronide, and 45 participants (56%) had detectable RPV N-glucuronide levels in their cervicovaginal fluid samples after injection (Bronx/Newark, USA $n=10$, Cape Town, South Africa $n=14$, Harare, Zimbabwe $n=21$) (Fig. 4C). We obtained 22 vaginal tissue samples from Bronx/Newark site after an intramuscular injection containing RPV and the metabolite,

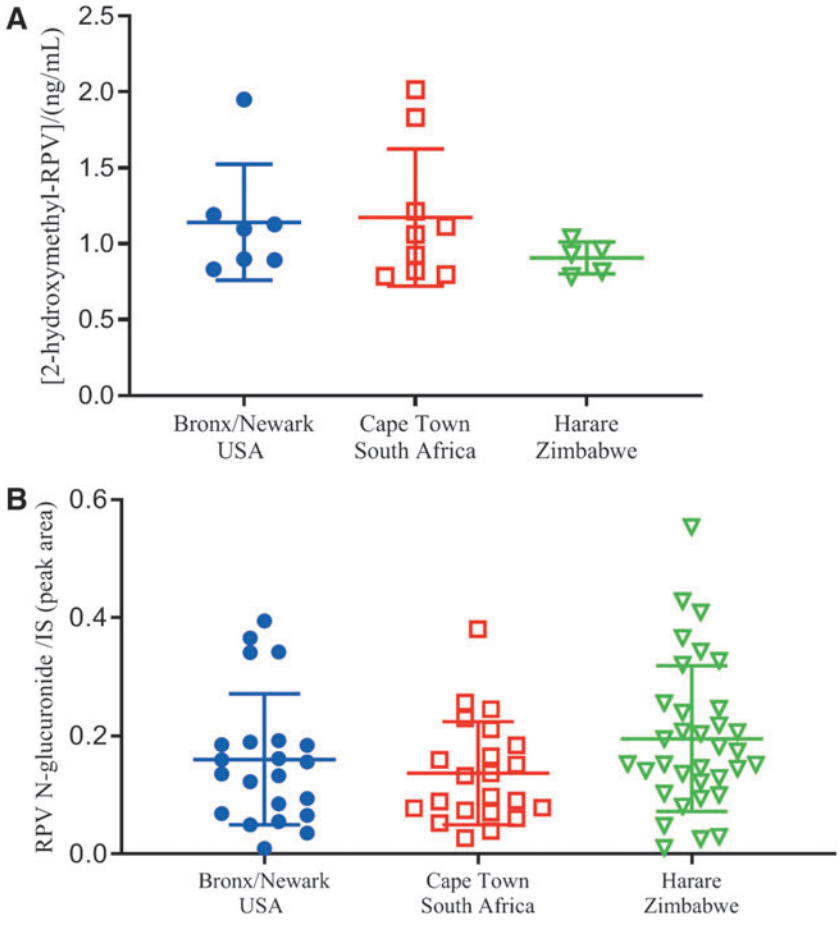

FIG. 2. Detection of 2-hydroxymethyl-RPV and RPV N-glucuronide in plasma samples of HTPN 076 research participants after an intramuscular injection containing RPV (1,200 mg of RPV was delivered in two 2 mL injections at 8-week intervals). **(A)** 2-hydroxymethyl-RPV and **(B)** RPV N-glucuronide in plasma samples of HPTN 076 study participants were detected by using an ultra-high-performance liquid chromatography-tandem mass spectrometry assay, as previously published.[9] The 2-hydroxymethyl-RPV metabolite was quantified by using a synthetic standard, and the levels of 2-hydroxymethyl-RPV are represented as ng/mL. Due to the lack of a synthetic standard for RPV N-glucuronide, data are represented as a peak area ratio to the IS, RPV-d6. A total of 80 plasma samples collected from study sites, Bronx/Newark, USA $n=22$, Cape Town, South Africa $n=24$, Harare, Zimbabwe $n=34$ were analyzed.

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.

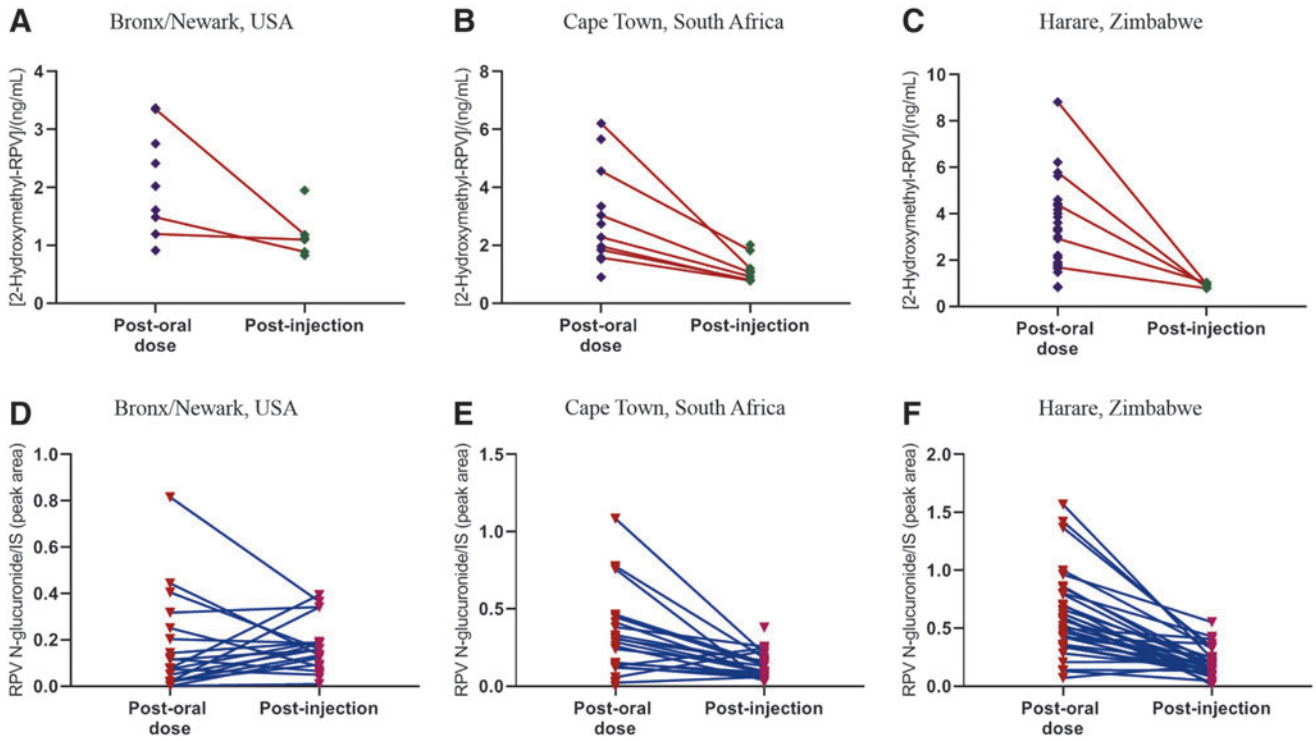

FIG. 3. Variation of the plasma levels of RPV metabolites, 2-hydroxymethyl-RPV, and RPV N-glucuronide in study participants at post-oral dose versus post-injection. Levels of 2-hydroxymethyl-RPV in plasma samples of HPTN 076 participants from **(A)** Bronx/Newark, USA, **(B)** Cape Town, South Africa, and **(C)** Harare, Zimbabwe after oral dosing versus injection. RPV N-glucuronide levels in plasma samples collected from **(D)** Bronx/Newark, USA, **(E)** Cape Town, South Africa, and **(F)** Harare, Zimbabwe after oral dosing versus injection. Eighty-three plasma samples collected from study sites, Bronx/Newark, USA $n=23$, Cape Town, South Africa $n=25$, Harare, Zimbabwe $n=35$ were analyzed for post-oral dose whereas 80 plasma samples collected from study sites, Bronx/Newark, USA $n=22$, Cape Town, South Africa $n=24$, Harare, Zimbabwe $n=34$ were analyzed for post-injection.

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.

RPV N-glucuronide was detected in 16 vaginal tissue samples (73%) from 16 unique participants (Bronx/Newark, USA $n=16$) (Fig. 4D).

Next-generation sequencing of participant genomic DNA

To determine whether genetic differences could be identified that might govern the observed interindividual variability in metabolite levels, we isolated genomic DNA from the participants and designed a targeted amplicon-based assay to sequence the exonic regions of *CYP3A4*, *CYP3A5*, *UGT1A1*, and *UGT1A4*. To gain a comprehensive understanding of the genetic variation within the study population, we genotyped all study participants, including those that did not receive RPV. Using this approach, we successfully sequenced 135 of the 136 participants (Bronx/Newark, USA $n=36$, Cape Town, South Africa $n=48$, Harare, Zimbabwe $n=51$). For one participant, we were not able to isolate high enough quality genomic DNA to carry out sequencing.

Targeted sequencing of CYP3A4 *and* CYP3A5

For *CYP3A4* (Table 2), four missense variants, all of which have been previously reported in the dbSNP database [as denoted by the RefSNP (rs) number], were detected: rs72552799 (R130Q), rs4986907 (R162Q, *CYP3A4*15A*), rs57409622 (R162W), and rs113667357 (Q200H). These variants were of relatively low frequency, with rs72552799 (R130Q) carried in one participant (Bronx/Newark, USA $n=1$), rs4986907 (R162Q, *CYP3A4*15A*) detected in six participants (Bronx/Newark, USA $n=2$, Cape Town, South Africa $n=1$, Harare, Zimbabwe $n=3$), rs57409622 (R162W) carried by one participant (Harare, Zimbabwe $n=1$), and rs113667357 (Q200H) carried by two participants (Cape Town, South Africa $n=2$). The observed frequencies of these variants in this study were 0.01, 0.04, 0.01, and 0.02, respectively. The functional impact of each of these variants is unknown.

For CYP3A5 targeted sequencing (Table 2), one missense variant rs142823108 (I149T) and one frameshift variant rs41303343 (*CYP3A5*7*, T346Y) were detected. The rs142823108 (I149T) variant was carried by two participants (Harare, Zimbabwe $n=2$), each heterozygous, for an observed frequency of 0.02. The rs41303343 (*CYP3A5*7*, T346Y) allele was present at a higher observed frequency of 0.24, as it was detected in 33 participants (Bronx/Newark, USA $n=2$, Cape Town, South Africa $n=16$, Harare, Zimbabwe $n=15$), with 2 of these being homozygous (observed frequency 0.02). The *CYP3A5*7* allele results in nonfunctional CYP3A5 protein[13]; however, we did not observe an impact of the *CYP3A5*7* genotype on RPV metabolism as the concentrations of 2-hydroxymethyl-RPV were

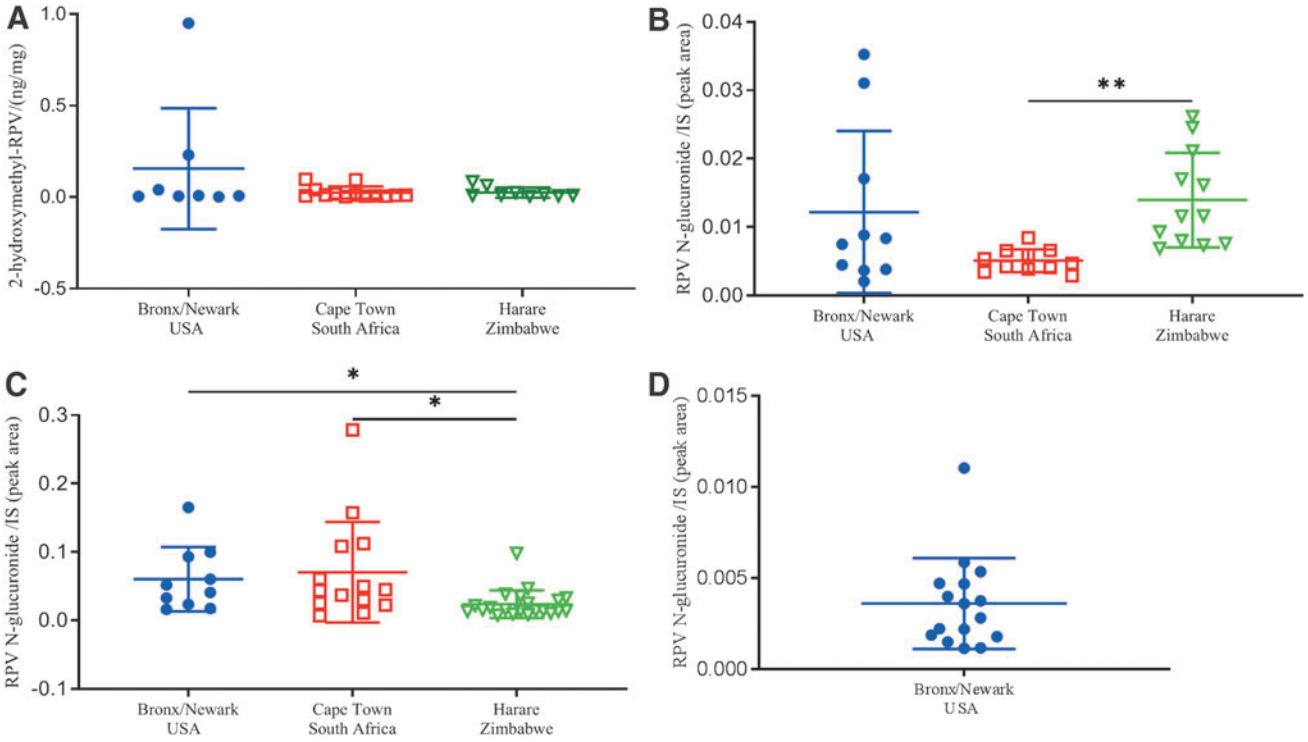

FIG. 4. Detection of RPV metabolites, 2-hydroxymethyl-RPV, and RPV N-glucuronide in rectal fluid, cervicovaginal fluid, and vaginal tissue samples of HTPN 076 research participants after RPV delivery via an intramuscular injection. **(A)** Detection of 2-hydroxymethyl-RPV in rectal fluid samples. For this, 79 rectal fluid samples from study sites Bronx/Newark, USA $n=21$, Cape Town, South Africa $n=23$, Harare, Zimbabwe $n=35$ were analyzed. The 2-hydroxymethyl-RPV metabolite was quantified by using a synthetic standard, and the levels of 2-hydroxymethyl-RPV are represented as ng/mg of sample. Detection of RPV N-glucuronide in **(B)** rectal fluid ($n=79$), **(C)** cervicovaginal fluid (80 samples: Bronx/Newark, USA $n=21$, Cape Town, South Africa $n=24$, Harare, Zimbabwe $n=35$), and **(D)** vaginal tissue (22 samples from Bronx/Newark, USA), samples using an ultra-high-performance liquid chromatography-tandem mass spectrometry assay as previously published.[9] RPV N-glucuronide data are represented as a peak area ratio to the IS, RPV-d6. Statistical significance was denoted as follows: $*p \le .05$; $**p \le .01$.

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.

3.0 ± 1.6 ng/mL versus 3.3 ± 1.7 ng/mL for participants carrying no *CYP3A5*7* alleles versus those carrying *CYP3A5*7* alleles.

Targeted sequencing of UGT1A1 *and* UGT1A4

One nonsynonymous variant and one upstream promoter insert variant was detected for UGT1A1 in the HPTN 076 participants, and these were variant rs144217005 (V109A) and rs34983651 (*UGT1A1*28*), respectively (Table 3). Variant rs144217005 was detected in 1 participant (Harare, Zimbabwe $n=1$) for an observed frequency of 0.01, whereas rs34983651 (*UGT1A1*28*) was carried by 22 (Bronx/Newark, USA $n=3$, Cape Town, South Africa $n=10$, Harare, Zimbabwe $n=9$) participants, resulting in an observed frequency of 0.16, and 5 of these individuals were homozygous (0.04 frequency). This allele, rs34983651 (*UGT1A1*28*), has been demonstrated to impair the transcription of *UGT1A1*, resulting in decreased protein expression and activity[14]; however, all of the participants carrying this allele had detectable levels of RPV N-glucuronide.

A total of eight missense variants of UGT1A4 were detected, and observed frequencies within the study population were calculated (Table 3). The highest frequency variant was rs45540231 (I176F). Twenty-six participants were heterozygous for this variant (Bronx/Newark, USA $n=2$, Cape Town, South Africa $n=13$, Harare, Zimbabwe $n=11$) for a frequency of 0.19, whereas one participant was homozygous (Harare Zimbabwe $n=1$) for a frequency of 0.01. This participant was the only individual that was homozygous for any *UGT1A4* variant. Of note, the levels of RPV N-glucuronide for this individual fell within the range of the other participants. The variant rs2011425 (*UGT1A4*3b*, L48V) showed the second highest frequency, as it was detected in 17 participants (Bronx/Newark, USA $n=6$, Cape Town, South Africa $n=4$, Harare, Zimbabwe $n=7$) for a frequency of 0.13. Variant rs3892221 (*UGT1A4*4*, R11W) was detected in seven participants (Bronx/Newark, USA $n=2$, Harare, Zimbabwe $n=1$, Cape Town, South Africa $n=4$) for a frequency of 0.05. The rs146073833 (G158R) variant was carried by seven participants (Bronx/Newark, USA $n=2$, Cape Town, South Africa $n=3$, Harare, Zimbabwe $n=2$) for an overall frequency of 0.05. The variant rs6755571 (*UGT1A4**2, P24T) was detected in four of the participants (Bronx/Newark, USA $n=2$, Cape Town, South Africa $n=1$, Harare, Zimbabwe $n=1$), and the frequency was 0.03. The other variants were rs141408391 (A58V) carried by one participant (Harare, Zimbabwe $n=1$), rs201935850 (K73N)

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.

Table 2. *CYP3A4* and *CYP3A5* Variants Detected in HPTN 076 Participants

Gene	dbSNP	Variant	Star allele	Amino acid mutation	*Bronx/Newark, USA* (n=*36*)		*Cape Town, South Africa* (n=*48*)		*Harare, Zimbabwe* (n=*51*)	
					n/*36*	n	n/*48*	n	n/*51*	n
CYP3A4	rs72552799	c.389G>A	—	R130Q	0.03 (Het)	1 (Het)	0	0	0	0
	rs4986907	c.485G>A	*CYP3A4*15A*	R162Q	0.06 (Het)	2 (Het)	0.02 (Het)	1 (Het)	0.06 (Het)	3 (Het)
	rs57409622	c.484C>T	—	R162W	0	0	0	0	0.02 (Het)	1 (Het)
	rs113667357	c.600A>T	—	Q200H	0	0	0.04 (Het)	2 (Het)		
CYP3A5	rs142823108	c.446T>C	—	I149T	0	0	0	0	0.04 (Het)	2 (Het)
	rs41303343	c.1035dupT	*CYP3A5*7*	T346Y Frameshift	0.06 (Het)	2 (Het)	0.33 (Het)	16 (Het)	0.26 (Het)	13 (Het)
									0.04 (Hom)	2 (Hom)

dbSNP designations are shown for all variants detected. Allele with star (*) assignments are noted as are the resulting amino acid sequence changes. The number of heterozygous (Het) and homozygous (Hom) individuals for each variant and site are noted. Observed frequencies for each variant are shown.

Table 3. *UGT1A1* and *UGT1A4* Variants Detected in HPTN 076 Participants

Gene	dbSNP	Variant	Star allele	Amino acid mutation	*Bronx/Newark, USA* (n=*36*)		*Cape Town, South Africa* (n=*48*)		*Harare, Zimbabwe* (n=*51*)	
					n/*36*	n	n/*48*	n	n/*51*	n
UGT1A1	rs34983651	(TA)7	*UGT1A1*28*	—	0.06 (Het)	2 (Het)	0.14 (Het)	7 (Het)	0.16 (Het)	8 (Het)
					0.03 (Hom)	1 (Hom)	0.06 (Hom)	3 (Hom)	0.02 (Hom)	1 (Hom)
	rs144217005	c.326T>C		V109A	0	0	0	0	0.02 (Het)	1 (Het)
UGT1A4	rs3892221	c.31C>T	*UGT1A4*4*	R11W	0.06 (Het)	2 (Het)	0.08 (Het)	4 (Het)	0.02 (Het)	1 (Het)
	rs6755571	c.70C>A	*UGT1A4*2*	P24T	0.06	2 (Het)	0.02 (Het)	1 (Het)	0.02 (Het)	1 (Het)
	rs2011425	c.142T>G	*UGT1A4*3b*	L48V	0.17 (Het)	6 (Het)	0.08 (Het)	4 (Het)	0.14 (Het)	7 (Het)
	rs141408391	c.173C>T		A58V	0	0	0	0	0.02 (Het)	1 (Het)
	rs201935850	c.219A>C		K73N	0	0	0	0	0.02 (Het)	1 (Het)
	rs146073833	c.472G>A		G158R	0.06 (Het)	2 (Het)	0.06 (Het)	3 (Het)	0.04 (Het)	2 (Het)
	rs45540231	c.526A>T		I176F	0.06 (Het)	2 (Het)	0.27 (Het)	13 (Het)	0.22 (Het)	11 (Het)
									0.02 (Hom)	1 (Hom)
	rs138822211	c.667A>C		I223L	0.03 (Het)	1 (Het)	0	0	0.04 (Het)	2 (Het)

dbSNP designations are shown for all variants detected. Allele with star (*) assignments are noted as are the resulting amino acid sequence changes. The number of heterozygous (Het) and homozygous (Hom) individuals for each variant and site are noted. Observed frequencies for each variant are shown.

carried by one participant (Harare, Zimbabwe $n=1$), and rs138822211 (I223L) carried by three participants (Bronx/Newark, USA $n=1$, Harare, Zimbabwe $n=2$) for frequencies of 0.01, 0.01, and 0.02, respectively.

Discussion

HPTN 076 was a phase II study that investigated the safety and tolerability of long-acting RPV in HIV-uninfected women across four research sites in Africa and the United States: Cape Town, South Africa; Harare, Zimbabwe; Bronx/Newark, USA.[10] In the current study, the metabolism of long-acting RPV was characterized in subjects who received intramuscular injections containing RPV (four intramuscular injections at eight-week intervals). In addition, the genetic variation in the genes that encode RPV metabolizing enzymes was investigated.

In our study, we detected RPV N-glucuronide and a hydroxylated metabolite of RPV, 2-hydroxymethyl-RPV, in plasma samples of subjects after oral administration of RPV. This is consistent with our previous report that RPV N-glucuronide, formed by UGT1A4, is the primary RPV plasma metabolite.[9] Somewhat surprisingly, we also detected plasma RPV N-glucuronide in 97.5% (78/80) of individuals after intramuscular injection. We detected 2-hydroxymethyl RPV in 90% (72/80) of participants. Orally administered drugs undergo first-pass hepatic metabolism since the liver contains high concentrations of P450s, UGTs, and other drug-metabolizing enzymes that are responsible for biotransformation. Previously, it has been reported *in vitro* that CYP3A4 and CYP3A5 are primarily responsible for RPV metabolism in liver.[9] It is known that enzymes in the CYP3A subfamily are highly abundant in liver.[15] Thus, CYP3A enzymes (CYP3A4/CYP3A5) in the liver may, indeed, play a primary role in the formation of 2-hydroxymethyl-RPV *in vivo*.

In our previous oral study, we found that two O-linked glucuronide conjugates of oxygenated metabolites of RPV also circulate in plasma to a greater extent than unconjugated metabolites, including 2-hydroxymethyl RPV; however, in the current study, these O-linked conjugates were not detectable after oral RPV administration or injection. These data suggest that the half-life of RPV N-glucuronide may be longer than other RPV metabolites and/or that this N-glucuronidation of RPV could be carried out by extrahepatic organs such as the kidney.

Currently, the overwhelming majority of what is known about the glucuronidation of drugs is within the context of oral drug administration. It is often assumed that since drugs delivered via alternative routes such as intramuscular injections largely bypass first-pass (hepatic) metabolism, metabolites will not be detectable or contribute to the pharmacology of the injectable drug. Our data here demonstrate that glucuronidation can be readily detected after injection of a long-acting drug that is known to undergo metabolism when administered orally. Further, the primary metabolite formed with both oral administration and intramuscular injection was the same. Future experiments are required to identify the source of RPV N-glucuronidation after intramuscular injection; however, it is possible that the glucuronidation could occur within the kidney.[16] The expression of UGTs is highly variable, and these enzymes are expressed across a range of tissues.[17] It has been reported that UGT1A9 and UGT2B7 are the major UGTs expressed in the kidney.[18] In addition, many UGTs, including UGT1A5, UGT1A6, UGT1A7, and UGT2B4, are known to be expressed in the kidney at low levels.[18] Interestingly, using a sensitive, nanobore liquid chromatography coupled to the tandem mass spectrometry approach, Harbourt *et al.* reported that expression of UGT1A4 is higher in the kidney than in the liver.[19] This finding along with our data from this study suggests that renal glucuronidation could be involved in the metabolism of long-acting injectable RPV.

We investigated the genetic variation of drug-metabolizing enzymes. Through previous work characterizing the human biotransformation of RPV, we identified *CYP3A4*, *CYP3A5*, *UGT1A1*, and *UGT1A4* as candidates for examination.[9] From these analyses, we detected several previously reported variants for *CYP3A4*, *CYP3A5*, *UGT1A1*, and *UGT1A4*. Many of these variants are of unknown function. The coverage threshold implemented in our analyses was in line with the American College of Medical Genetics and Genomics clinical laboratory standards for next-generation sequencing.[20] From these genotypic analyses, we detected eight missense genetic variants for UGT1A4. To date, several variants of *UGT1A4* have been studied, including *UGT1A4*2, UGT1A4*4,* and *UGT1A4*3b*.[21] Among these variants, UGT1A4 L48V (*UGT1A4*3b*) is known to lead to increased rates of glucuronidation.[22] However, we did not find a correlation between the UGT1A4 genotype and RPV N-glucuronide levels in our study. Of note, we did not observe an overlap in the participants with undetectable levels of N-glucuronide RPV after an injection or oral dosing. Each of the participants exhibited detectable levels of N-glucuronide RPV in at least one of the dosing phases. Taken together, our data suggest that multiple UGT enzymes may contribute to long-acting RPV metabolism in the context of injection, and that the enzymes responsible for RPV metabolism may differ depending on the route of administration.

We detected one frameshift variant in *CYP3A5*, *CYP3A5*7*, and we found that carrying this allele had no impact on RPV metabolism. It is known that this shift introduces a premature termination codon at position 348.[13] Based on the previous studies, CYP3A5*7 occurs at a frequency of about 8% in the African population.[23] Interestingly, this variant has not been found in Asian or White populations.[24–26] Our findings suggest that RPV hydroxylation is not exclusively or primarily carried out by CYP3A5.

In this work, as a major finding we detected RPV N-glucuronide in rectal fluid, cervicovaginal fluid, and vaginal tissue samples collected from HPTN 076 participants after an intramuscular injection containing RPV. This is an interesting finding as to the best of our knowledge, glucuronidated metabolites have not been previously reported in cervicovaginal fluid or vaginal tissue after *in vivo* dosing. Elimination routes for glucuronides include urinary, biliary, and intestinal excretion, and glucuronidated metabolites are often found in feces.[27] This likely explains why we were able to detect RPV N-glucuronide in rectal fluid samples of the study participants. In addition, we detected RPV N-glucuronide in vaginal tissue samples. However, in our work, we were not able to quantitate RPV N-glucuronide levels due to the lack of a synthetic standard. Interestingly,

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.

our laboratory has previously shown the expression of P450s, including CYP3A4 and CYP3A5 in vaginal tissues; however, in the current work, we did not observe any oxidative metabolites of RPV in vaginal tissue.[28] A study of the expression of transporters and drug-metabolizing enzymes in vaginal tissue has been reported.[29] The 2-hydroxymethyl-RPV metabolite was present in the rectal fluid of certain subjects; however, we did not detect the glucuronide conjugate of 2-hydroxymethyl-RPV. The mechanisms that govern the metabolite profiles we observed are unclear; however, since we did not observe any correlations between genetic variants and RPV metabolite levels or distributions, the observed interindividual differences in RPV metabolite levels may be due to transport mechanisms and involvement of multiple metabolism pathways. Notably, our current analysis was limited to females. In the future, it would be of interest to investigate potential sexual dimorphisms in RPV metabolism.

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.

Conclusions

In conclusion, we detected the same two RPV metabolites, 2-hydroxymethyl-RPV and RPV N-glucuronide in the plasma samples of study participants after both oral and intramuscular RPV. Interestingly, RPV N-glucuronide was also detected in rectal fluid, cervicovaginal fluid, and vaginal tissue samples postinjection. These data suggest that glucuronidation may be the primary route of RPV metabolism after injection. To date, the pharmacological activities or toxicities of these metabolites are unknown. To investigate potential genetic variation in RPV metabolism, we isolated genomic DNA and performed next-generation sequencing of *CYP3A4*, *CYP3A5*, *UGT1A1*, and *UGT1A4*. However, we did not find a correlation between the detected variants and RPV metabolite levels. Interindividual differences that we observed in RPV metabolite levels are likely due to involvement of multiple metabolism pathways and other mechanisms such as transport. Taken together, our results provide novel insights into the metabolism of long-acting RPV as well as a unique foundation to understand the pharmacokinetics of injectable drugs more broadly.

Authors' Contributions

This article was written by the contributions of all the authors. All authors have given approval to the final version of the article.

Acknowledgments

The authors thank Dr. Z Mike Chirenje at the University of Zimbabwe–University of California, San Francisco (UZ-UCSF) Collaborative Research Programme, Harare, Zimbabwe for his contributions to the HPTN 076 study.

Author Disclosure Statement

No competing financial interests exist.

Funding Information

This work was supported by the HIV Prevention Trials Network (HPTN) sponsored by the National Institute of Allergy and Infectious Diseases (NIAID), National Institute on Drug Abuse, National Institute of Mental Health, and Office of AIDS Research, of the NIH, DHHS (UM1 AI068613).

References

1. Garvey L, Winston A: Rilpivirine: A novel non-nucleoside reverse transcriptase inhibitor. Expert Opin Investig Drugs 2009;18:1035–1041.
2. Azijn H, Tirry I, Vingerhoets J, *et al.*: TMC278, a next-generation nonnucleoside reverse transcriptase inhibitor (NNRTI), active against wild-type and NNRTI-resistant HIV-1. Antimicrob Agents Chemother 2010;54:718–727.
3. Janssen PA, Lewi PJ, Arnold E, *et al.*: In search of a novel anti-HIV drug: Multidisciplinary coordination in the discovery of 4-[[4-[[4-[(1E)-2-cyanoethenyl]-2,6-dimethylphenyl] amino]-2-pyrimidinyl]amino]benzonitrile (R278474, rilpivirine). J Med Chem 2005;48:1901–1909.
4. Baert L, van 't Klooster G, Dries W, *et al.*: Development of a long-acting injectable formulation with nanoparticles of rilpivirine (TMC278) for HIV treatment. Eur J Pharm Biopharm 2009;72:502–508.
5. Jackson A, McGowan I: Long-acting rilpivirine for HIV prevention. Curr Opin HIV AIDS 2015;10:253–257.
6. Almazroo OA, Miah MK, Venkataramanan R: Drug metabolism in the liver. Clin Liver Dis 2017;21:1–20.
7. Benedetti MS, Whomsley R, Poggesi I, *et al.*: Drug metabolism and pharmacokinetics. Drug Metab Rev 2009;41: 344–390.
8. Mulder GJ: Glucuronidation and its role in regulation of biological activity of drugs. Annu Rev Pharmacol Toxicol 1992;32:25–49.
9. Lade JM, Avery LB, Bumpus NN: Human biotransformation of the nonnucleoside reverse transcriptase inhibitor rilpivirine and a cross-species metabolism comparison. Antimicrob Agents Chemother 2013;57:5067–5079.
10. Tolley EE, Li S, Zangeneh SZ, *et al.*: Acceptability of a long-acting injectable HIV prevention product among US and African women: Findings from a phase 2 clinical Trial (HPTN 076). J Int AIDS Soc 2019;22:e25408.
11. Bekker LG, Li S, Pathak S, *et al.*: Safety and tolerability of injectable Rilpivirine LA in HPTN 076: A phase 2 HIV pre-exposure prophylaxis study in women. EClinicalMedicine 2020;21:100303.
12. Whirl-Carrillo M, McDonagh EM, Hebert JM, *et al.*: Pharmacogenomics knowledge for personalized medicine. Clin Pharmacol Ther 2012;92:414–417.
13. Lamba J, Hebert JM, Schuetz EG, Klein TE, Altman RB: PharmGKB summary: Very important pharmacogene information for CYP3A5. Pharmacogenet Genomics 2012; 22:555–558.
14. Rauchschwalbe SK, Zuhlsdorf MT, Schuhly U, Kuhlmann J: Predicting the risk of sporadic elevated bilirubin levels and diagnosing Gilbert's syndrome by genotyping UGT1A1*28 promoter polymorphism. Int J Clin Pharmacol Ther 2002;40:233–240.
15. de Wildt SN, Kearns GL, Leeder JS, van den Anker JN: Cytochrome P450 3A: Ontogeny and drug disposition. Clin Pharmacokinet 1999;37:485–505.
16. Knights KM, Rowland A, Miners JO: Renal drug metabolism in humans: The potential for drug-endobiotic interactions involving cytochrome P450 (CYP) and UDP-glucuronosyltransferase (UGT). Br J Clin Pharmacol 2013;76:587–602.

17. Margaillan G, Rouleau M, Fallon JK, *et al.*: Quantitative profiling of human renal UDP-glucuronosyltransferases and glucuronidation activity: A comparison of normal and tumoral kidney tissues. Drug Metab Dispos 2015;43:611–619.
18. Knights KM, Miners JO: Renal UDP-glucuronosyltransferases and the glucuronidation of xenobiotics and endogenous mediators. Drug Metab Rev 2010;42:63–73.
19. Harbourt DE, Fallon JK, Ito S, *et al.*: Quantification of human uridine-diphosphate glucuronosyl transferase 1A isoforms in liver, intestine, and kidney using nanobore liquid chromatography-tandem mass spectrometry. Anal Chem 2012;84:98–105.
20. Rehm HL, Bale SJ, Bayrak-Toydemir P, *et al.*: ACMG clinical laboratory standards for next-generation sequencing. Genet Med 2013;15:733–747.
21. Benoit-Biancamano MO, Adam JP, Bernard O, *et al.*: A pharmacogenetics study of the human glucuronosyltransferase UGT1A4. Pharmacogenet Genomics 2009;19:945–954.
22. Gulcebi MI, Ozkaynakcı A, Goren MZ, Aker RG, Ozkara C, Onat FY: The relationship between UGT1A4 polymorphism and serum concentration of lamotrigine in patients with epilepsy. Epilepsy Res 2011;95:1–8.
23. Lee SJ, Usmani KA, Chanas B, *et al.*: Genetic findings and functional studies of human CYP3A5 single nucleotide polymorphisms in different ethnic groups. Pharmacogenetics 2003;13:461–472.
24. Hustert E, Haberl M, Burk O, *et al.*: The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics 2001;11:773–779.
25. van Schaik RH, van der Heiden IP, van den Anker JN, Lindemans J: CYP3A5 variant allele frequencies in Dutch Caucasians. Clin Chem 2002;48:1668–1671.
26. Park SY, Kang YS, Jeong MS, Yoon HK, Han KO: Frequencies of CYP3A5 genotypes and haplotypes in a Korean population. J Clin Pharm Ther 2008;33:61–65.
27. Yang G, Ge S, Singh R, *et al.*: Glucuronidation: Driving factors and their impact on glucuronide disposition. Drug Metab Rev 2017;49:105–138.
28. To EE, Hendrix CW, Bumpus NN: Dissimilarities in the metabolism of antiretroviral drugs used in HIV preexposure prophylaxis in colon and vagina tissues. Biochem Pharmacol 2013;86:979–990.
29. Zhou T, Hu M, Cost M, Poloyac S, Rohan L: Short communication: Expression of transporters and metabolizing enzymes in the female lower genital tract: implications for microbicide research. AIDS Res Hum Retroviruses 2013; 29:1496–1503.

Address correspondence to:
Namandje N. Bumpus
Department of Pharmacology and Molecular Sciences
Johns Hopkins University School of Medicine
725 North Wolfe Street
Biophysics 307
Baltimore, MD 21205
USA

E-mail: nbumpus1@jhmi.edu

Downloaded by 71.227.122.194 from www.liebertpub.com at 03/26/21. For personal use only.