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Tenofovir and tenofovir disoproxil fumarate pharmacokinetics from intravaginal rings

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Abstract

Objectives—To compare the distribution of tenofovir in sheep vaginal lumen, tissue, and plasma following topical delivery of the antiretroviral drug from intravaginal rings, either as tenofovir or the disoproxil fumarate prodrug.

Design—Comparative pharmacokinetic study in sheep.

Method—Intravaginal rings formulated to achieve equivalent release rates of tenofovir and its disoproxil fumarate prodrug were evaluated for 28 days in sheep, with four animals in each group. Drug concentrations were measured by high-performance liquid chromatography–mass spectrometry.

Results—Tenofovir levels in cervicovaginal lavage were indistinguishable ($P > 0.30$) in both groups, but tissue levels in animals receiving the prodrug were 86-fold higher than those receiving tenofovir, and approximately 50 times higher than the level shown to be protective of HIV infection in the CAPRISA 004 trial.

Conclusion—This is the first study to compare the pharmacokinetics of tenofovir and its disoproxil fumarate prodrug administered topically to the vaginal tract. These in-vivo data show that the prodrug leads to significantly higher drug tissue levels than tenofovir, a finding that may have important implications for the development of preexposure prophylaxis strategies based on topical delivery of antivirals to the female genital tract.

Keywords

intravaginal ring; preexposure prophylaxis; sheep; tenofovir; tenofovir disoproxil fumarate; topical delivery

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Conflicts of interest

Several authors (A.M.M., E.K., C.N., J.G., I.B., R.A.W., T.J.S.) are current or past employees of Auritec Pharmaceuticals Inc., and therefore have financial competing interests, as per *AIDS* guidelines. These include ownership of stocks or shares, paid employment, board membership, active patent applications, and research grants.

Introduction

For decades, attempts to develop an effective preexposure prophylaxis (PrEP) strategy for the prevention of HIV transmission have failed, despite the high priority given to these initiatives. Results from two recent clinical trials are renewing optimism in the potential of PrEP [1,2]. The Centre for the AIDS Program of Research in South Africa 004 was a double-blind study of 889 uninfected heterosexual women in South Africa [3]. Women who were instructed to use a pericoital 1% tenofovir (TFV) gel showed a 39% reduction in HIV transmission compared with those receiving placebo gel, whereas a 54% reduction in transmission was observed in women who had higher adherence rates. In an attempt to increase adherence and achieve coital independence, a number of groups, including ours, are attempting to develop an intravaginal ring (IVR) that delivers TFV [4–6].

Tenofovir disoproxil fumarate (TDF; Viread; Sinoway International, Jiangsu, China) a prodrug of TFV, was developed because the parent drug has low oral bioavailability [7]. TDF also exhibits a significant increase in cellular uptake compared with TFV in tissue culture [8]. As a result, we hypothesized that local TFV tissue levels resulting from intravaginal delivery would be significantly higher for TDF-containing IVRs compared with those delivering TFV.

Methods

Production of intravaginal rings

Silicone pod IVRs were produced according to methods described elsewhere [6] using compacted drug cores (16 mg each, two per ring) of either TFV or TDF, coated with poly-D,L-lactide (PLA), and with 1 mm diameter delivery channels.

Study design

In-vivo studies were performed with four sheep that received TFV rings and four that received TDF rings. Additional details are presented under Supplemental Digital Content, <http://links.lww.com/QAD/A200>.

Samples collection, processing, and analysis

Vaginal rings were inserted on day 0 and plasma and cervicovaginal lavage (CVL) samples were collected [6] at time points shown in Fig. 1. Vaginal tissue samples were obtained by biopsy during necropsy on day 28 from regions spanning the cervix to the introitus. Samples were processed, stored, and transported using standard methods [6]. Bioanalysis was performed by high-performance liquid chromatography–mass spectrometry using methods described under Supplemental Digital Content, <http://links.lww.com/QAD/A200>; Lower limits of quantitation: CVL, 5 ng/ml; plasma, 10 ng/ml; tissue, 2 ng/g. The run-to-run coefficient of variation [9] for all methods was below 10%.

In the studies involving TDF IVRs, drug levels were analyzed in terms of TFV, mono(POC)TFV (POC = isopropylloxycarbonyloxymethyl), and bis(POC)TFV and were converted to total TFV on a molar basis.

Results

The IVRs exhibited sustained release *in vitro* with pseudo-zero order kinetics controlled by the polymer coating and delivery channel size [5,6]: cumulative release of TFV 66.8 ± 5.1 μg per day ($n = 6$) and TDF 64.0 ± 7.1 μg per day ($n = 6$).

The prodrug TDF is formulated as the fumarate salt of the bis(POC) phosphonate ester of TFV. The hydrolytic lability of the phosphonate ester moieties results in reversion of TDF to TFV in aqueous solution: $\tau_{1/2} = 8$ h at pH 7.4, 37°C [10]. All analytical measurements involving TDF were made in terms of bis(POC)TFV, mono(POC)TFV, and TFV on a molar basis and converted to total TFV in ng/ml or ng/g depending on the compartment sampled. Over 90% of the residual drug in the used TDF IVRs was in the bis(POC)TFV form, whereas the drug was primarily present as TFV in CVL (89%) and tissue (91%).

The sheep has been developed as a cost-effective large mammal animal model for studying vaginal toxicity and pharmacokinetics of topically administered microbicides [6,11]. The measured CVL and tissue TFV levels over the course of the 28-day sheep study are shown in Fig. 1. Levels of TFV in CVL from both IVR formulations were constant (TFV, mean 196 ± 125 ng/ml; TDF, mean 155 ± 143 ng/ml) and indistinguishable ($P > 0.30$) for the duration of the 28 days (Fig. 1, circles). Note that CVL levels represent a dilution of the drug concentrations present in the vaginal lumen. Mean TFV levels in the vaginal tissue at day 28 from the TDF IVRs were 86 times higher ($P < 0.001$) than those from the corresponding TFV IVRs (TFV IVRs, mean 39 ± 42 ng/mg; TDF IVRs, mean 3340 ± 2009 ng/mg). Measurements of total tissue TFV levels are susceptible to surface contamination of the biopsy from residual lumen [12], but this effect was minimized by taking appropriate precautions during sampling and by collecting multiple biopsies from each animal's vaginal tract. Plasma levels were below the 10 ng/ml level of quantitation throughout the study. Residual drug analysis on the used IVRs showed that the TFV rings delivered $18 \mu\text{mol}$ (5.2 ± 1.0 mg) of TFV, whereas the TDF rings delivered $15 \mu\text{mol}$ (4.2 ± 0.8 mg) of TFV over the course of the 28-day study.

Discussion

The present study demonstrates that IVRs releasing TFV and TDF at equivalent rates in sheep over 28 days led to dramatically different distributions of TFV in vaginal tissue. The residual drug in vaginal lumen from TDF-releasing rings was mostly present as free TFV, suggesting that any bis(POC)TFV rapidly partitioned into the tissue, wherein it was hydrolyzed to TFV, an observation that is supported by our drug measurements in these compartments. A pharmacokinetic model comparing the distribution of TFV delivered from a 1% gel [13] to our TDF IVR is presented in Fig. 2.

The mode of action of nucleotide analogue reverse transcriptase inhibitors (NRTIs) such as TFV in preventing HIV infection is elegantly described by Hendrix *et al.* [12] in their six-compartment pharmacokinetic–pharmacodynamic (PK–PD) interaction model. This model links drug and viral levels in vaginal lumen, tissue, blood, and the associated CD4⁺ cells to the associated pharmacodynamic outcomes. For TFV PrEP in the female genital tract, it appears that CD4⁺ cells in vaginal tissue correspond to the pharmacokinetic compartment most likely to determine seroconversion outcomes [14]. Within the CD4⁺ cells, TFV undergoes anabolism to the fully phosphorylated active moiety (TFVpp), not measured here due to the resource-intensive nature of the assays and the preliminary nature of this report. The low intrinsic inhibitory potential of TFV [15] suggests that, in the absence of dose-related toxicity, higher TFV levels will result in better protection from HIV infection [14]. Recently, it has been shown that women with TFV cervicovaginal fluid concentrations greater than 1000 ng/ml were significantly protected from HIV infection [13]. The corresponding TFV tissue levels were not reported, but the mean TFV concentrations in vaginal secretions were 14 times higher than the mean tissue levels when both compartments were sampled [13]. Using this relationship, the above protective levels in lumen correspond to approximately 70 ng/g in vaginal tissues. In our studies, sheep that received a TDF ring

had TFV tissue levels approximately 50 higher than this protecting limit, whereas the sheep receiving the TFV rings had tissues levels approximately half of this threshold level.

The hydrolytic instability of TDF has precluded its use in gels [14], but the prodrug remains intact in the pod IVR platform discussed here, even after 28 days of use. It is premature to speculate on the relative merits of IVR versus gel delivery technology for local PrEP in preventing women from becoming infected with HIV. However, we believe that data presented here suggest a theoretical superiority of local delivery of the TDF prodrug over the TFV parent drug in the search for a well tolerated and effective HIV PrEP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The production and in-vitro testing of the IVRs was carried out by E.K., C.N., J.G., and I.B. under the supervision of J.A.M. Animal studies were carried out by K.L.V. and M.M., and were coordinated by A.M.M. and R.A.W. The bioanalysis was carried out by S.K. The data were analyzed by J.A.M., M.M.B., A.M.M., and T.J.S. M.M.B. and T.J.S. were responsible for study oversight and manuscript preparation. A.M.M., R.A.W., and J.A.M. contributed to manuscript editing.

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Fig. 1. Mean (SD) pharmacokinetic profile of tenofovir and tenofovir disoproxil fumarate delivered topically via intravaginal rings over 28 days in sheep

Cervicovaginal lavage drug levels (circles, mean + SD) obtained using intravaginal ring (IVR) formulations containing tenofovir (TFV) (blue, $n = 4$) and tenofovir disoproxil fumarate (TDF) (red, $n = 4$), reported as total TFV. Sheep vaginal tissue drug levels (triangles, mean + SD) obtained using IVR formulations containing TFV (blue, $n = 2$, four sampling locations per animal) and TDF (red, $n = 4$, nine sampling locations per animal), reported as total TFV.

Fig. 2. Pharmacokinetic four-compartment model illustrating the partitioning of tenofovir/tenofovir disoproxil fumarate delivered to the female genital tract from topical formulations (a) A 1% tenofovir (TFV) gel: a decreasing concentration gradient, from the gel to plasma, occurs across all compartments. Drug partitioning is mostly unidirectional (large solid arrows), with broken arrows indicating theoretically possible drug flux that is unlikely in practice. The concentration ranges as a function of compartment type were reported by Karim *et al.* [13]: median levels of 100 000 ng/ml in cervicovaginal lumen and 7000 ng/g in vaginal tissue; color depth is illustrative of TFV levels. (b) Tenofovir disoproxil fumarate (TDF) intravaginal ring (IVR): the distribution of TDF and TFV is significantly different than in (a). Here, TDF partitions from the IVR into the cervicovaginal lumen, wherein the drug is stable at the expected pH [10]; a small amount of hydrolysis to TFV occurs (vertical arrow). Lumen TDF diffuses into the vaginal tissue, wherein it accumulates and hydrolyzes to TFV. The tissue TFV acts as a depot and diffuses back into the lumen (large orange arrow). The concentration ranges as a function of compartment type are shown and correspond to median TFV levels of 7000 ng/ml in cervicovaginal lumen, assuming a 60-fold dilution in the CVL [6] and 3000 ng/g in vaginal tissue. Broken arrows indicate theoretically possible drug flux that is unlikely in practice; color depth is illustrative of TDF (red) and TFV (blue) levels.