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Pharmacokinetic evaluation of tenofovir disoproxil fumarate released from an intravaginal ring in pig-tailed macaques after 6 months of continuous use

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Abstract

A reservoir intravaginal ring (IVR) eluting tenofovir disoproxil fumarate (TDF) was evaluated for 6 months of continuous use in normally cycling female pigtailed macaques with monthly IVR exchanges to define pharmacokinetics and safety. Tenofovir levels in vaginal secretions and tissue remained consistent for six months with no adverse safety concerns.

Keywords

HIV microbicide; pre-exposure prophylaxis; non-human primates; sustained release

Introduction

Although some pre-exposure prophylaxis modalities, in particular Truvada™ (oral TDF, the prodrug of tenofovir, in combination with emtricitabine) and topical tenofovir (TFV) gels, have been shown to be effective in reducing acquisition of HIV, it is pivotal to develop different delivery platforms to increase effectiveness by providing sustained topical drug delivery to overcome poor adherence. [1, 3, 10, 25, 26]. Intravaginal rings (IVR) have been used as a vaginal delivery device for contraception, hormone replacement therapy and treatment of endometriosis, dysmenorrhea, and polycystic ovarian syndrome [4, 5, 8, 20]. The capacity of IVRs to provide sustained and controlled release of drugs over extended periods of time have recently been exploited to deliver antiretrovirals (ARV) [7, 12, 13, 18, 27]. The high costs associated with large-scale phase II/III clinical trials require us to fully

evaluate the performance of the drug delivery systems in animal models for pharmacokinetics, safety and potential for prevention of mucosal immunodeficiency virus transmission. Here the nonhuman primate model has been the model of choice [14, 17, 19, 24]. Although nonhuman primate efficacy studies against vaginal virus transmission using an IVR are limited [2, 9, 22] we recently demonstrated complete protection against 16 once-weekly vaginal exposures of SHIV162p3 in normally cycling female pigtailed macaques with a TDF reservoir IVR [23]. Because of sampling restrictions during the efficacy phase of the study, the study was extended for an additional 8 weeks after the last virus exposure to further define the pharmacokinetics and safety of the TDF IVR during 6 months of continuous use.

Materials and Methods

Animals

The animals were housed under biosafety level 2 containment conditions according to the *Guide for the Care and Use of Laboratory Animals* (National Research Council of the National Academies, 2010) at the Centers for Disease Control and Prevention (CDC). The CDC Animal Care and Use Committee approved all procedures outlined in the study. TDF reservoir IVR (130 ± 10 mg TDF and 20 ± 2 mg NaCl per IVR) were inserted into normal cycling pig-tailed macaques ($n=6$) at week 0. The TDF IVR macaques and 12 untreated controls were exposed once weekly to SHIV162p3 for 16 weeks [23]. The IVRs were replaced monthly, two days after virus exposure, at weeks 4, 8, 12, 16 and 20, with the final IVR removed at week 24.

Pharmacokinetics and Safety

Vaginal secretions were obtained with Weck-Cel sponges during the challenge phase at the monthly IVR changes (weeks 4–16) and once weekly with a multistwab device for the follow-up period (weeks 19–24). Cervicovaginal lavages (CVL) of the genital tract were collected during the follow-up period. Vaginal biopsies were obtained at weeks 20, 21, and 23. TDF and TFV were quantitated by liquid chromatography-mass spectrometry in vaginal secretions and tissues [16, 19]. Analysis of mucosal inflammation was done by the measurement of cytokines using a Milliplex™ MAP (Millipore) fluorescent multiplexed bead-based assay as previously described [14, 21].

Data Analysis

Change in cytokines and chemokines over time were examined using Friedman tests of log transformed values followed by Wilcoxon signed-rank tests with false discovery rate (FDR) adjusted p -values for post-hoc pairwise comparisons between each of 8 time points (wk 19 – 24.5) and wk –1 and wk 0, respectively.

Results and Discussion

The TDF-IVR macaques ($n=6$) remained both viral RNA negative and seronegative after 16 weekly SHIV exposures while 11/12 control macaques were infected after a median of four exposures [23]. Mucosal TDF concentrations were highly variable in the follow-up phase of

the study (data not shown), most likely due to the instability of TDF in an aqueous environment, but were consistent with levels previously described [23]. High median mucosal concentrations of TFV, 1.8×10^5 (range, $1.1 \times 10^4 - 6.6 \times 10^5$) ng/mL, were found in vaginal fluids collected at the monthly IVR exchange. During the follow-up phase, median TFV concentrations were 1.2×10^5 ($7.5 \times 10^0 - 2.1 \times 10^6$) ng/mL (Fig 1B). Although the distribution of drug in the vaginal vault was consistent ($10^4 - 10^6$ ng/mL), TFV levels at the upper end of the range were more frequently detected proximally than distally to the placement of the IVR during the challenge and the follow up phase.

A CVL of the macaque genital tract with 5 mL of PBS is approximately a 1:10 dilution of vaginal secretions [6]. TFV concentrations in CVL (Fig 1C) appeared very stable during the follow up phase, 6.4×10^3 ng/mL ($1.9 \times 10^2 - 1 \times 10^5$) and were found to be above the 1000 ng/mL that was effective in preventing HIV acquisition in women using a 1% TFV gel [15]. Although a modest decline in TFV concentrations in CVL was observed 3 days after ring removal, these remained above the 1000 ng/mL protective threshold (Fig 1C).

The vaginal biopsy concentrations of TFV were also high with median of 1.3×10^4 ($8.1 \times 10^2 - 9.0 \times 10^4$) ng/g tissue (Figure 1D). These levels are substantially higher than 113 ng/mL in vaginal tissue reported in the CAPRISA-004 trial. The tissue TFV concentrations and the cumulative *in vivo* release of 64 ± 11 mg in 28 days (determined from residual drug in IVR post- removal) mirrored our previous observations [23].

The induction of mucosal inflammation due to IVR presence continuously for 6 months was monitored by measurement of 18 vaginal cytokines and chemokines (Table 1). Although there were some differences in cytokines pre-ring placement compared to post-ring, when using FDR adjusted p - values these differences reflected variability between time points (wk 19–24.5), suggesting that the effects are not due to ring placement, and in another study we found that there was no difference between TDF and placebo rings in vaginal cytokine and chemokine concentrations (manuscript in preparation). These findings show that extended presence of the IVR does not alter the cytokine/chemokine patterns in the local mucosa, although other subclinical toxic effects due to the drug or the IVR cannot be deciphered by the above data [11, 14, 21]

This is the first study to report the extended presence of an IVR in macaques and shows that it can safely deliver TDF mucosally at protective concentrations in this model. A phase 1 study was initiated in December, 2013 (28).

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Figure 1. Study design and drug levels in vaginal secretions and tissues

1A: TDF reservoir IVR were inserted into normal cycling pig-tailed macaques (n=6) and the animals were exposed to sixteen once-weekly vaginal exposures of SHIV162p3. The IVRs were exchanged monthly for an additional eight weeks (follow-up). Vaginal secretions were collected during the monthly IVR changes (weeks 4–16) and once weekly during the follow-up period (weeks 19–24). Vaginal tissues were obtained on weeks 20, 21 and 23.

1B: TFV concentrations in vaginal fluid obtained proximal (grey bars) and distal (white bars) to IVR placement during the monthly IVR exchanges and the follow-up period. The bar

corresponds to the minimum and maximum concentrations at each time point with a line at the median.

1C: TFV concentrations in CVL obtained during the follow-up period. The bar corresponds to the minimum and maximum concentrations at each time point with a line at the median.

1D: TFV concentrations in vaginal tissue obtained proximal (grey bars) and distal (white bars) to IVR placement during the follow-up period. The bar corresponds to the minimum and maximum concentrations at each time point with a line at the median.

Table 1
Cytokine/chemokine concentrations (pg/mL) in vaginal secretions from the TDF IVR treatment group (N=6)

Cytokine/ chemokine ^I	Baseline (week)		TDF IVR treatment (week)- median (range)							Post IVR
	-1	0	19	20	21	21.5	22	23	24	24.5
IL-1 β	466.1 (1.85-5,450)	87.26 (33.1-351.5)	564.6 (183.9-1,438)	365 (68.59-6,735)	264.1 (91.45-12,202)	2,734 (419-14,779)	2,047 (28.15- 3,794)	1,556 (382-5,314)	235.5 (81.72-6,425)	1,359 (340.7-5,676)
IL-1Ra	22,167 (6,490-46,232)	17,310 (4,261-35,757)	26,042 (5,788-74,237)	46,239 (22,721-69,629)	17,844 (8,294-76,480)	67,511 (17,506-78,784)	35,374 (13,814-66,647)	46,877 (10,743-65,401)	45,865 (20,785-77,097)	50,872 (16,424-89,300)
G-CSF	67.79 (38.59-328.7)	95.86 (5.49-452.9)	786.2 (176.2-6,365)	220.2 (72.5-537)	165.1 (87.2-1,400)	375.7 (55.87-1,018)	175.3 (6.43-711.8)	176.3 (68.93-826.9)	284.3 (118.1-951.2)	216.7 (51.95-923.1)
IFN γ	26.2 (2.68-38.65)	13.75 (1.5-77.93)	59.6 (9.47-102.5)	52.4 (18.95-114.2)	35.34 (13.2-789.9)	169.5 (29.72-298.5)	67.44 (25.72-265.4)	71.39 (23.29-118.5)	53.03 (21.9-407.9)	66.34 (24.86-96.46)
IL-2	19.5 (6.03-33.56)	5.94 (1.73-17.73)	39.85 (24.43-52.16)	38.28 (22.56-97.21)	16.74 (6.14-67.76)	80.68 (21.43-166.1)	60.92 (1.14-91.16)	68.44 (42.67-132.3)	33.78 (10.61-123.1)	77.69 (28.65-135.5)
IL-4	38.59 (18.03-178.8)	45.76 (27.23-683.7)	57.44 (15.58-193.1)	61.82 (18.75-185.3)	34.06 (5.01-1,074)	124.2 (84.11-657.9)	42.65 (16.14-864)	43.76 (16.42-297.9)	117.9 (11.69-200.8)	35.48 (14.75-75.84)
IL-6	17.92 (2.11-67.16)	43.24 (5.49-402.8)	387 (10.39-5,116)	25.69 (5.26-87.75)	133.1 (2.97-1,143)	98.57 (16.67-1,004)	44.5 (17.43-52.09)	74.38 (5.09-356.8)	164.7 (4.96-592.3)	68.3 (23.56-159.9)
IL-12/23p40	39.76 (12.07-99.07)	21.31 (9.13-61.89)	110 (22.18-381.9)	61.63 (18.35-156)	25.55 (4.89-65.21)	61.13 (17.43-231.1)	50.42 (11.15-107.2)	58.63 (22.97-93.85)	143 (17.74-198.3)	30.18 (10.36-100.3)
IL-13	51.54 (16.95-60.68)	19.26 (5.49-108.9)	50.09 (33.83-103.3)	45.15 (38.36-66.88)	19.47 (7.3-90.59)	87.25 (56.87-139.7)	76.65 (26.01-93.64)	60.46 (45.05-124.3)	59.36 (35.04-91.05)	80.85 (48.86-103)
IL-15	51.71 (23.47-149.8)	29.97 (9.98-158.7)	91.59 (33.27-329.8)	162.1 (17.45-815.7)	57.81 (28.44-106.3)	129.2 (46.34-688.7)	140.5 (57.16-236.6)	131.4 (50.93-242.3)	143 (28.35-835)	142.3 (22.1-687.2)
IL-17	17.5 (0.58-37.17)	5.09 (2.38-14.67)	13.26 (3.86-25.19)	9.03 (1.35-16.61)	1.96 (1.29-6.18)	17.59 (4.72-50.84)	13.24 (1.89-28.5)	6.45 (2.02-23.83)	11.56 (1.31-16.42)	7.43 (1.31-13.13)

Cytokine/ chemokine ^I	Baseline (week)		TDF IVR treatment (week)- median (range)								Post IVR	
	-1	0	19	20	21	21.5	22	23	24	24.5		
MCP-1	597.1 (79.46-2,503)	779.1 (73.41-1,978)	849.1 (209.2-1,015)	505.9 (163.7-1,459)	701 (61.26-1,998)	1,533 (505.4-4,660)	1,040 (285.1-1,852)	1,147 (307.1-2,216)	793.8 (302.4-1,619)	854.8 (342-2,193)		
MIP-1 β	59.34 (15.77-440.8)	34.6 (14.59-80.18)	26.57 (3.93-73.65)	40.45 (12.18-61.35)	18.05 (4.07-58.77)	55.16 (17.43-316.8)	29.26 (4.38-120.8)	48.02 (7.95-118.2)	31.9 (2.53-77.7)	64.25 (17.74-89.65)		
sCD40L	66.43 (5.94-129.3)	48.14 (5.49-248.7)	360.1 (29.62-2,107)	100.1 (10.23-1496)	76.74 (9.72-548.8)	88.44 (30.15-798.5)	110.8 (30.15-2,274)	77.26 (9.89-881)	330.4 (86.08-1,221)	94.47 (30.68-903.4)		
TNF- α	172.2 (57.6-1,135)	83.42 (5.49-376.3)	819.3 (272.2-1,469)	582.3 (18.35-963.2)	181.3 (18.66-6,267)	973.6 (296.5-2,907)	1,257 (73.45-1,818)	968.1 (219-1,306)	704.2 (17.74-1,884)	922.6 (312.2-1,882)		
VEGF	288.4 (46.54-1,462)	147.8 (5.76-775)	705.6 (323.3-1,303)	767.2 (512.4-1,426)	414.1 (52.92-1,936)	2,612 (576.5-4,885)	968.6 (427-1,211)	871.8 (195.3-1,823)	1,103 (334.9-2,441)	880.6 (334.9-3,475)		
IL-18	4,229 (468.4-36,174)	928 (39.34-87,726)	1,697 (485.8-3,998)	2,092 (364.3-11,118)	1,287 (344.4-3,265)	4,435 (789.1-6,466)	1,521 (644.2-6,449)	3,725 (515.8-5,363)	1,680 (327-14,218)	1,824 (639.2-24,084)		
GM-CSF	2.38 (1.16-4.95)	3.9 (1.15-119.6)	20.04 (2.17-72.12)	3.89 (2.11-88.81)	19.5 (0.08-112.8)	17.26 (0.76-196.8)	13.59 (4.96-26.13)	9.73 (0.29-385)	21.66 (7.69-41.06)	14.22 (3.93-909.9)		

^I Friedman tests found differences over time in VEGF, IL-1ra, IFN γ , IL-2, IL-12/23p40, IL-13, IL-17 and sCD40L. FDR adjusted p-values for post-hoc pairwise comparisons between each of 8 time points (wk 19 - 24.5) and wk -1 and wk 0, suggest changes in these cytokines (except IL-2) were due to differences among time points (wk 19 -25) signifying that the effects are not due to ring placement.