

**Combination emtricitabine and tenofovir disoproxil fumarate prevents vaginal SHIV infection in macaques harboring *C. trachomatis* and *T. vaginalis***

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## ABSTRACT

Genital inflammation associated with STIs increases susceptibility to HIV but it is unclear if the increased risk can reduce the efficacy of pre-exposure prophylaxis (PrEP). We investigated if co-infection of macaques with *Chlamydia trachomatis* and *Trichomonas vaginalis* decreases the prophylactic efficacy of oral FTC/TDF. Macaques were exposed to SHIV vaginally each week for up to 16 weeks and received placebo or FTC/TDF peri-coitally. All placebos were infected with SHIV while 4/6 PrEP-treated animals remained uninfected ( $p=0.03$ ). Oral FTC/TDF maintains efficacy in a macaque model of STI coinfection, although the infection of 2 macaques signals a modest loss of PrEP activity.

## BACKGROUND

PrEP with FTC/TDF is a novel HIV prevention strategy that was approved by the FDA in 2012. Although studies have generally shown that drug concentrations are highly predictive of protection [1], it is not known if the efficacy of PrEP can be diminished in subpopulations at higher risk of HIV infection due to, for instance, co-infection with other STIs. *Chlamydia trachomatis* (CT) and *Trichomonas vaginalis* (TV) are among the most prevalent STIs and have been associated with an increased risk of HIV acquisition in women [2]. Both STIs can modulate local immune factors and cause an influx of immune cells to the vaginal mucosa. These biological changes likely contribute to the increased HIV risk and also raise questions about their effect on the effectiveness of PrEP. For instance, a high density of immune cells at the vaginal mucosa might conceivably require more drug coverage of susceptible target cells and alter drug protection thresholds. Likewise, cellular activation increases intracellular dNTP concentrations which could reduce the antiviral activity of FTC-TP and TFV-DP.

The effect of CT/TV on PrEP in women cannot accurately be assessed from subgroup analysis in clinical trials because baseline prevalence of CT/TV infection is generally low and participants are frequently tested and treated for their STIs. Given the difficulty of addressing this question clinically, during trials or PrEP implementation, alternative experimental approaches such as animal models may help assess the effect of STIs on PrEP under well-controlled experimental conditions. Here we integrated a repeat exposure macaque SHIV transmission model with a model of co-infection with CT and TV to assess potential effects of CT/TV on PrEP with FTC/TDF.

## MATERIALS AND METHODS

### *Macaques*

All animal procedures were approved by the CDC IACUC. Pig-tailed macaques were housed at the CDC under the care of CDC veterinarians in accordance with the Guide for the Care and Use of Laboratory Animals.

### *CT and TV infection*

Because these infections can self-resolve in humans and macaques, animals were re-inoculated every 3-4 weeks to ensure maintenance of the STIs. Figure 1 shows the experimental design and the weekly STI infection status. CT serovars D (strain D-LC, provided by H. Caldwell) or E (strain UW/5) were alternated between inoculations to best represent predominantly prevalent serovars of genital *Chlamydia* infection.

### *Efficacy of FTC/TDF in CT/TV-infected macaques*

The efficacy of FTC/TDF was evaluated using a repeat exposure SHIV transmission model previously described [3]. Macaques received FTC/TDF (20 and 22 mg/kg, respectively; n=6) or PBS (n=5) orally in a peri-coital modality consisting of one dose 24h before SHIV challenge and a second dose 2h thereafter. Animals were considered protected if they remained seronegative and negative for SHIV RNA and DNA during the 16 weekly virus challenges and the following 14 weeks of drug washout [3]. Infected animals in the PrEP-treated group were maintained on FTC/TDF until week 16. Risk for SHIV infection in animals receiving PrEP or placebo was compared using a Wald test and randomization-based inference [4].

***Effect of CT/TV on the pharmacokinetic (PK) profile of TFV and FTC and on dATP and dCTP levels***

To understand the effects of inflammation on cellular dNTP levels in vaginal tissues, we measured dATP and dCTP levels in vaginal biopsies collected, as previously described [5], from 5 macaques SHIV-infected in a previous study. Samples were collected prior to and 1, 2 and 3 weeks following inoculation with CT/TV using methods previously described [3]. Macaques also received a single oral dose of FTC/TDF at week 1 to define the PK profile of FTC and TFV and tissue drug penetration in the presence of STIs. Analytes were measured as previously described [6].

## **RESULTS**

***Efficacy of FTC/TDF in macaques infected with CT/TV***

After 16 SHIV challenges, only 2/6 PrEP-treated animals (DC42 and BB495) were infected while all 5 placebo controls were infected after a median of 2 SHIV exposures (Figure 1B). Infection in PrEP-treated macaques was significantly less likely than in animals receiving placebo ( $p=0.03$ ). Based upon infection rates per exposure, the efficacy of FTC/TDF in STI-infected macaques (91% [95% CL=56.3-98.7]) was not different to that previously seen in animals that also received FTC/TDF PrEP but were not co-infected with STIs (100% [95% CL=54.1-100];  $p=0.69$ , conditional logistic regression model 2-way interaction term) [3]. The average peak viremia in the placebo animals was  $2.8 \times 10^6$  copies/mL compared to  $3.5 \times 10^4$  and  $2.2 \times 10^4$  copies/mL in DC42 and BB495. Neither DC42 nor BB495 developed K65R, K70E or M184V (not shown). Absolute SHIV RNA levels in vaginal and rectal secretions were reduced

in PrEP breakthrough infections compared to placebo ( $p=0.02$  and  $p=0.03$ , respectively), although the number of weeks with detectable shedding did not differ among the two groups (Suppl. Figure 1).

### ***Intracellular concentrations of TFV-DP, FTC-TP and dNTP in infected and protected animals***

We compared TFV-DP, FTC-TP, dATP/TFV-DP and dCTP/FTC-TP in PBMCs at the estimated week of infection among PrEP breakthrough infections BB495 and DC42 and the 4 protected animals (Suppl. Figure 2). The concentrations of TFV-DP in BB495 and DC42 were 13 and 31 fmols/ $10^6$  cells compared to 14-22 fmols/ $10^6$  cells in protected animals ( $p=1.000$ ). Likewise, the concentrations of FTC-TP in DC42 and BB495 at infection were 188 and 168 fmols/ $10^6$  cells compared to 160-318 fmols/ $10^6$  cells in protected animals ( $p=1.000$ ). The dATP/TFV-DP ratios in DC42 and BB495 at infection (2.1 and 7.3, respectively) were similar to those seen in protected animals (4.0 to 4.5,  $p=1.000$ ) as were the dCTP/FTC-TP ratios ( $p=0.518$ , Suppl. Figure 2).

### ***Effect of STIs on drug PK and dNTP concentrations in vaginal tissues***

Mean dATP and dCTP concentrations in vaginal biopsies prior to CT/TV infection were  $1.56 \times 10^4$  and  $0.34 \times 10^4$  fmols/g, respectively, compared to  $3.28 \times 10^4$  and  $1.00 \times 10^4$  fmols/g after CT/TV infection ( $p=0.02$  for both comparisons, Figure 2). One FTC/TDF dose administered to CT/TV-infected macaques resulted in median FTC-TP and TFV-DP levels in vaginal tissues at 24h of 21.5 (3.8-36.7) and 3.7 (1.0-7.4) fmols/mg, respectively. The overall PK profiles for FTC and TFV in vaginal secretions and plasma were similar to that previously seen in STI-naïve animals (Suppl. Figure 3) [3].

## DISCUSSION

We investigated in a macaque model the hypothesis that PrEP efficacy might be reduced in women with increased risk of HIV acquisition due to STI coinfection. Our experimental design used a validated PrEP model incorporating clinically relevant FTC/TDF pharmacokinetics, repeated SHIV exposures to model populations at high-risk of HIV infection, and increased per-exposure SHIV infection risk due to CT/TV infection [3, 7, 8]. We show that the efficacy of FTC/TDF was not significantly different from that previously seen in macaques without these two STIs [3], although the infection of 2 macaques points to a modest loss of PrEP activity. This preservation of protection was consistent with a recent sub-analysis of Partner PrEP participants in which either one of the partners had an STI within 3 months of study visit. In this high HIV incidence subpopulation, TDF and FTC/TDF had consistently high efficacy against HIV acquisition [9]. Although these findings were from a small number of participants and included both men and women, they help support our observations in the macaque model and suggest that PrEP use in populations with increased risk for HIV acquisition retains efficacy.

Our design using repeated SHIV challenges over several months required boosting doses of CT/TV to ensure maintenance of the STIs. The resolution of CT or TV in some animals and the re-inoculation mimics the natural clinical course in women who are infected, diagnosed and treated, and may later become re-infected after subsequent exposures. This approach was highly effective as the majority of animals maintained both STIs during the course of the study. However, the STI status may have been in some instances incorrect due to insufficient specimen collection on the vaginal swabs. Nevertheless, the detection of CT/TV infection over the

majority (>80%) of the study weeks demonstrates the robustness of this experimental approach and is also in line with earlier studies with pig-tailed macaques [10, 11].

Although the protection conferred by FTC/TDF was statistically significant, two of the macaques became infected with SHIV during PrEP. In earlier studies, the same FTC/TDF dose and modality fully protected STI-naïve animals including those treated with DMPA [3, 12]. We measured TFV-DP and FTC-TP in PBMCs at the estimated week of infection and found no differences between infected and protected animals. The two infected macaques also had reduced genital virus shedding compared to untreated animals which provided evidence for mucosal drug penetration and local antiviral activity. Our data showing no effect of CT/TV on drug penetration into the vaginal mucosa further suggest little or no effect of CT/TV on mucosal drug pharmacokinetics. These findings do not clearly point to pharmacologic differences as an explanation of both PrEP failures, although we did notice that FTC-TP levels in vaginal biopsies were lower than those previously reported in STI-uninfected macaques [3]. This observation requires confirmation with additional animals using identical biopsy collection methods.

Although CT/TV are non-ulcerative, they can induce cervical petechiae while *Chlamydia* can also cause mucosal perturbations such as cervical erosion or ectropion [13, 14]. Disruptions in tissue integrity may potentially facilitate virus entry and access to target cells in the lamina propria. Unfortunately, our study design did not incorporate colposcopic evaluations to understand if more severe clinical signs of STI infection might account for the two PrEP breakthrough infections. Infection of these animals might be related to tissue inflammation and recruitment and activation of HIV target cells due to CT/TV infection, which may provide an expanded source of highly permissive cells that may be harder to protect by PrEP [8, 15]. Elevated dATP and dCTP concentrations in vaginal tissues supports cellular activation which

may have reduced but not eliminated the antiviral activity of TFV-DP and FTC-TP given the observed reduction in SHIV shedding. The high vaginal dATP and dCTP levels seen after STI infection, although confounded by SHIV infection and concurrent administration of FTC/TDF, which may potentially alter dNTP pools [6], points to potential modulations of drug activity due to inflammation. Unfortunately, we did not evaluate if higher mucosal inflammation markers were associated with SHIV acquisition in the 2 PrEP-treated animals.

This study had several limitations. First, our analysis was done with non-ulcerative STIs and the mucosal barrier may be less compromised than with other ulcerative STIs such as genital herpes or syphilis. In addition, only vaginal biopsies were collected for mucosal drug and dNTP measurements, whereas *C. trachomatis* preferentially infects the columnar mucosa of the cervix. Also, although the analysis of infection rates per exposure did not demonstrate differences in efficacy of FTC/TDF among STI-infected and uninfected macaques, our study was not powered to evaluate differences in efficacy at the subject level. Finally, we modeled a peri-coital regimen containing only 2 weekly doses of FTC/TDF. It is possible that daily FTC/TDF could have had a slightly better efficacy because of intracellular FTC-TP and TFV-DP accumulation after daily dosing.

In summary, we show that a peri-coital FTC/TDF regimen is effective in macaques co-infected with CT/TV, although the infection of two animals signals a modest loss of PrEP activity. Our analysis suggests a possible role for inflammation and cellular activation in PrEP failure, and highlights the need for further studies with other STIs to better define potential modulations of PrEP activity.

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Disclaimer: The findings and conclusions of this manuscript are those of the authors and do not necessarily represent the official views of the Centers for Disease Control and Prevention.

## **CONFLICT OF INTEREST DISCLOSURE STATEMENT**

JGGL and WH are named on a US government patent on inhibition of HIV infection through chemoprophylaxis. The other authors have no conflicts.

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## FIGURE LEGENDS

**Figure 1. Efficacy of PrEP with FTC/TDF in CT/TV-infected animals.** A, Study design including weeks of CT/TV inoculation (shaded columns), weeks of SHIV challenges (weeks 1 to 16), and weeks with positive (gray) or negative (black) CT/TV results. CT inocula ( $1 \times 10^6$  inclusion-forming units) was applied directly to the cervical mucosa; infection was monitored with the Aptima Combo 2 test [8]. TV (strain Balt-42;  $5 \times 10^6$  trichomonads) was propagated in culture and applied directly to the vaginal mucosa; infection was monitored with the in-Pouch culture system and Aptima Combo 2 test [8]. Of the 187 Aptima tests analyzed for CT status, 24 were negative for a 12.8% negativity rate. Similarly, 33 of 176 In-Pouch cultures were negative for TV, resulting in an 18.7% negative rate over the course of 16 weeks. B, Kaplan-Meier plot representing the cumulative percentage of uninfected macaques as a function of the number of weeks. We assumed an eclipse phase of 7 days to estimate time of infection from the first detection of SHIV RNA in plasma. Dotted lines denote the weeks of STI inoculation.

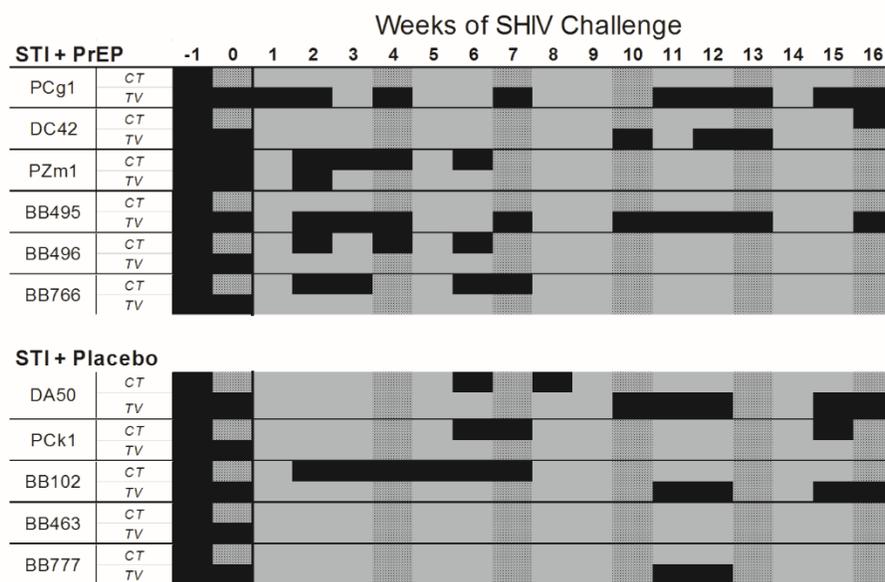
**Figure 2. Effect of CT and TV infection on dATP and dCTP concentrations in vaginal tissues.** Concentrations of dATP and dCTP in vaginal biopsies collected before (open circles) and after (grey circles) STI infection. Three to four 1.8-mm pinch biopsies were obtained from the vaginal wall prior to CT/TV co-infection, and then at weeks 1, 2, and 3. Levels of dATP, and dCTP were measured in tissue homogenates as described and compared using a mixed effect model after controlling for time trend following STI infection.

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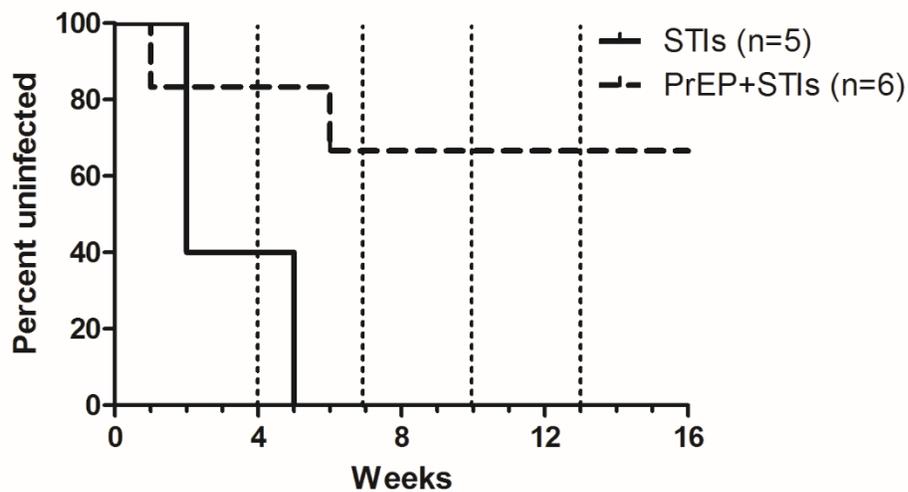
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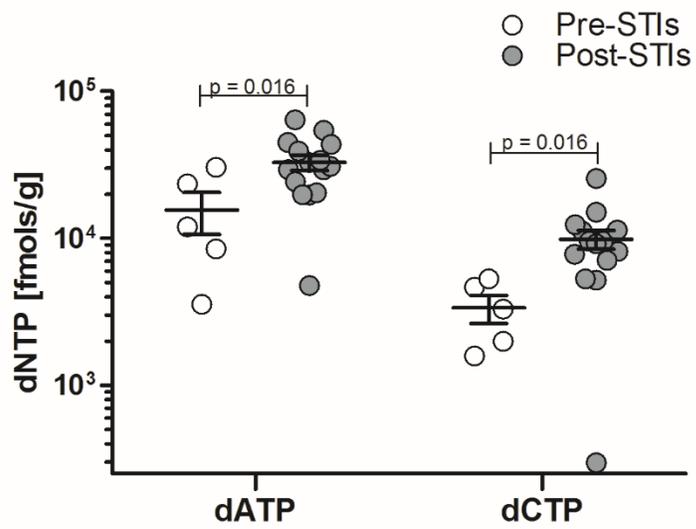
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