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Extended Postexposure Protection Against Vaginal Simian/Human Immunodeficiency Virus Infection With Tenofovir Alafenamide Fumarate/Elvitegravir Inserts in Macaques

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Abstract

Vaginal inserts that can be used on demand before or after sex may be a desirable human immunodeficiency virus (HIV) prevention option for women. We recently showed that inserts containing tenofovir alafenamide fumarate (TAF, 20 mg) and elvitegravir (EVG, 16 mg)

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Potential conflicts of interest.

J. G. G.-L. and W. H. are named in US government patents on “Inhibition of HIV infection through chemoprophylaxis” (US patents 9,044,509 B2; 9,579,333 B2; 9,937,191 B2 and 10,335,423 B2) and “HIV post-exposure prophylaxis” (US patent 11,191,763 B2), and the US government patent application on “HIV preexposure prophylaxis.” (US 2022/0265689 A1). M. M. P., V. A., G. F. D., and M. R. C. are named in patent applications US 2021/0379089 A1 on “Pharmaceutical compositions and methods of making on-demand solid dosage formulations,” inventions that were developed under a USAID-funded cooperative agreement. All other authors report no potential conflicts.

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Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

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were highly protective against repeated simian/human immunodeficiency virus (SHIV) vaginal exposures when administered to macaques 4 hours before or after virus exposure (93% and 100%, respectively). Here, we show in the same macaque model that insert application 8 hours or 24 hours after exposure maintains high efficacy (94.4% and 77.2%, respectively). These data extend the protective window by TAF/EVG inserts and inform their clinical development for on-demand prophylaxis in women.

Keywords

HIV preexposure and postexposure prophylaxis; macaque models; topical PrEP and PEP; tenofovir alafenamide; elvitegravir

Despite a global declining trend in the incidence of human immunodeficiency virus (HIV), about 1.5 million new infections were reported last year [1]. The majority of new HIV infections occur in sub-Saharan Africa, and adolescent girls and young women are at the core of the epidemic in this region, accounting for 69% of these infections [1]. Preexposure prophylaxis (PrEP) with antiretroviral drugs has become an essential tool to prevent HIV transmission. The PrEP toolbox for women now consists of 2 systemic options, including daily oral emtricitabine and tenofovir disoproxil fumarate and bimonthly injectable long-acting cabotegravir, and a monthly dapivirine vaginal ring [2]. Recent end-user preference studies in Africa reported the desire for topical and on-demand products that reduce systemic drug exposure. Using placebo products, young women have similar preferences for inserts, films, and vaginal rings (~30% for each option), but in South Africa, the inserts were the most preferred dosage form [3]. Providing a postexposure prophylaxis (PEP) option can increase acceptance, enhance compliance, and improve effectiveness by limiting the impact on sexual practices and improving user control.

CONRAD has developed fast-dissolving inserts containing tenofovir alafenamide fumarate (TAF, 20 mg) and elvitegravir (EVG, 16 mg) intended for on-demand use before or after vaginal or rectal sex [4]. In phase 1 clinical trials, TAF/EVG inserts demonstrated safety and favorable pharmacokinetics (PK) and pharmacodynamics after both vaginal (NCT03762772) and rectal (NCT04047420) applications [5, 6]. The combination of TAF and EVG provides a flexible, pharmacologically forgiving, on-demand PrEP or PEP option [4]. TAF is a potent prodrug of tenofovir (TFV) that improves uptake of TFV and loading of cells with tenofovir-diphosphate (TFV-DP) [7]. EVG is a strand transfer inhibitor that blocks HIV integration, a step that follows reverse transcription by several hours. Thus, adding EVG has an advantage for postexposure application and potential for extension of the PEP window [8, 9]. Indeed, PK studies in macaques supported a postexposure application by demonstrating rapid insert disintegration and tissue loading [10]. Vaginal tissue levels of EVG peaked at 2 hours with maximum concentration (C_{max}) values 6 times the protein-adjusted 95% inhibitory concentration. In contrast, tissue TFV-DP C_{max} was seen 24 hours postdosing [10].

We recently showed in a macaque model that TAF/EVG inserts were highly protective (92%–100% efficacy) when applied 4 hours before or 4 hours after repeated vaginal exposures to simian/human immunodeficiency virus (SHIV) [10]. In an extension of this

work, we assessed PEP efficacy at 8 and 24 hours to better inform selection of the dosing window for clinical development.

METHODS

Animals and Drugs

Twenty-one normally cycling female pigtailed macaques 5–16 years of age were used for PEP efficacy studies, with 6 in each treatment group and 9 placebo controls. Three SHIV-infected animals from the control group were used for a terminal PK study. All animal procedures were approved by the Centers for Disease Control and Prevention Institutional Animal Care and Use Committee (Supplementary Methods). Placebo and TAF/EVG inserts containing 22.4 mg of TAF (equivalent to 20 mg of tenofovir alafenamide free base) and 16 mg of EVG were supplied by CONRAD [4].

Vaginal PEP Efficacy of TAF/EVG Inserts and Insert PK

One TAF/EVG or placebo insert was placed in the vagina of the macaque close to the cervix (n = 6 per arm) 8 hours or 24 hours after each vaginal exposure to SHIV162P3 as described previously (ARP-6526, HIV Reagent Program, Manassas, Virginia; GenBank: KF042063) [10]. Animals were challenged once weekly for 13 weeks, as previously described [10]. Infection outcome was measured against the placebo control group consisting of 5 historical and 4 real-time controls (n = 2 for the 8-hour arm and n = 2 for the 24-hour arm). Details of sample collection and molecular and serologic testing of infection are described in the Supplementary Methods. Drug PK analysis in vaginal tissues was conducted in 3 SHIV-positive macaques that received inserts once a week for 4 consecutive weeks. Biopsies (n = 3 per animal) were collected at weeks 1 and 4.

Statistical Analysis

Kruskal–Wallis tests were used to determine differences in weight (kilograms) and age (months) of macaques between study arms [10, 11]. Efficacy and time to infection were calculated, and survival analysis was conducted with SAS PROC LIFETEST (SAS version 9.4 software). Viral load in infected animals was compared with the Wilcoxon rank-sum test. TFV-DP levels in vaginal tissue collected at weeks 1 and 4 were compared using quantile regression with SAS version 9.4 software and applying the bootstrap technique to resolve the correlation of data for statistical inference.

RESULTS

The window of PEP activity by TAF/EVG was investigated in 2 groups of macaques that received inserts 8 hours or 24 hours after vaginal SHIV challenges (Figure 1A). Both weight and age were not significantly different between study arms ($P = .60$ and $P = .85$, respectively, Kruskal–Wallis test). In the placebo arm, 8 of 9 animals became infected after 1 (n = 2), 2 (n = 2), 3 (n = 1), or 4 (n = 3) exposures (Figure 1B) with a median time to infection of 3 exposures. In the 8-hour PEP arm, 5 of 6 animals remained protected after 13 weekly exposures with 1 animal infected at exposure 11. The calculated efficacy of the 8-hour PEP was 94.41% (95% exact confidence interval [CI], 57.03%–99.27%]). In the

24-hour PEP arm, 3 of 6 animals became infected with 2 at exposure 2, and 1 at exposure 13, resulting in an efficacy of 77.23% (95% exact CI, 20.00%–93.52%). The log-rank test indicated a statistically significant difference in time to infection between the control arm and the 8-hour ($P = .004$) and 24-hour ($P = .02$) arms, but not between the 2 treated arms ($P = .49$). Supplementary Figure 1 shows the estimated time of infection relative to phase of the menstrual cycle and highlights how animals were infected at different phases of the cycle. To assess the pressure of continuous drug application on the development of infection, peak plasma viral load was compared between infected controls and the 4 breakthroughs (1 in the 8-hour arm and 3 in the 24-hour arm) (Figure 1C). There was some reduction in peak viral load in treated animals (TAF/EVG group: median, 8×10^5 [range, 2×10^5–8×10^6] copies/mL; controls: median, 9×10^7 [range, 4×10^5–1×10^9] copies/mL), although the difference was not statistically significant ($P = .14$, Wilcoxon rank-sum test). To evaluate possible emergence of drug resistance, 2 treated animals that had breakthrough infections continued receiving TAF/EVG inserts for 8 weeks after infection. Consistent with other studies with topical products in different dosage forms, breakthrough infections had no drug resistance viruses to TFV or EVG [12]. During the efficacy studies, we did not want to disturb the cervicovaginal environment to measure mucosal tissue drug levels; however, we measured TFV-DP concentrations in peripheral blood mononuclear cells (PBMCs) prior to each virus challenge, representing levels 1 week after the previous dosing. Consistent drug accumulation in PBMCs was not observed with weekly dosing (Supplementary Figure 2). We noted week-to-week fluctuations in TFV-DP detection 7 days after dosing within a given animal, with approximately 25% (range, 14%–93%) of exposures done without detectable TFV-DP in PBMCs (Supplementary Table 1). In samples with detectable TFV-DP, median levels in the 8-hour and 24-hour arms were 97 (range, 32–330) fmol/10^6 cells and 100 (range, 15–305) fmol/10^6 cells, respectively.

A terminal PK study was conducted in 3 SHIV-positive animals to define whether TFV-DP could also be detected in vaginal tissue 1 week after dosing and if those levels increased after multiple doses (Figure 2A). Earlier time points were previously studied and reported [10]. In this study, the median vaginal TFV-DP 1 week after dosing was 1125 (range, 105–2360) fmol/mg after 1 dose and 16 994 (range, below the limit of quantification to 4 787 162) fmol/mg after 4 weekly doses (Figure 2B). The difference in median levels after 1 and 4 insert applications was significant based on the quantile regression analysis ($P < .0001$); however, the number of animals was limited. Also, tissue TFV-DP levels decreased between 1 and 4 applications in 1 of 3 animals.

DISCUSSION

Poor adherence and early PrEP discontinuation limit the clinical and public health benefits of HIV PrEP [13]. Preference and acceptability studies in African women indicate no clear preference for a particular product, highlighting the need for multiple HIV prevention options for women [3]. Increased options may maximize uptake and increase adherence and continuation of PrEP [3, 14]. Topical inserts are discreet, portable, able to be self-administered, and have the potential to provide a flexible on-demand HIV prevention option that can be administered not only before, but also after sex [4]. In a recent phase 1 study in women, a single dose of TAF/EVG inserts applied vaginally was safe, well tolerated,

and acceptable [5, 6]. Furthermore, it demonstrated high levels of TFV-DP and EVG in cervicovaginal fluids and tissues, compatible with protection from HIV infection [5]. In nonhuman primates, TAF/EVG inserts showed robust biological efficacy (92%–100%) when administered vaginally as on-demand PrEP or PEP, 4 hours before or after SHIV challenges [10].

Determining the window of PEP activity by a vaginal insert intended for on-demand use can inform potential product efficacy depending upon time of application postexposure. We show that application 8 hours after SHIV exposure maintains high (94%) efficacy in macaques. However, increasing the gap between virus exposure and insert use to 24 hours reduced efficacy to 77%. These efficacy data, together with those from a previous study demonstrating 100% protection when inserts were administered 4 hours after sex [10], suggest an optimally effective PEP dosing window of up to 8 hours after sex, with somewhat reduced efficacy between 8 and 24 hours after exposure. This window, combined with the on-demand PrEP dosing option, provides product use flexibility, an attribute that is highly desirable for an on-demand, female-controlled HIV prevention method. While the exact contribution of EVG and TAF to the high PEP efficacy at 8 hours is not known, the rapid tissue uptake of EVG, coinciding with the first viral integration steps, may point to an important role of EVG in protection. We previously reported that EVG levels rise rapidly in tissue after vaginal application, remaining high for at least 4 hours before starting to decline to undetectable levels at 24 hours [10]. Concurrently, TFV-DP rises more slowly, achieving high levels in tissue (>1000 fmol/mg) 24 hours after insert application. Thus, it is possible to speculate that rapid high levels of EVG prevent virus integration, while sustained high levels of TFV-DP maintain a continued inhibition of HIV replication. Testing inserts containing TAF or EVG alone, however, may be needed to confirm the individual contributions of EVG and TAF to protection.

The analysis of TFV-DP concentrations 7 days after insert application documented inconsistent residual TFV-DP in PBMCs and accumulation of TFV-DP in vaginal tissues after repeated dosing. Thus, the role of long-lasting TFV-DP in protection remains unclear. Although our design cannot dissect the role of long-lasting TFV-DP in protection, some observations suggest that rather than residual drug concentrations from previous dosing, drug levels from TAF/EVG inserts applied as PEP early enough after virus exposure are a critical contributor to the observed protection. First, 2 breakthrough infections occurred after multiple doses and challenges, suggesting that tissue accumulation is not a factor. Tissue drug accumulation was also variable and inconsistent. Second, more infections occurred with 24-hour PEP than with 8-hour PEP, indicating that time of PEP is the limiting factor as previous dosing drug levels should have been similar. Third, TFV-DP in PBMCs as a surrogate at the time of virus exposure was not consistently detected in all animals, and some animals remained protected despite no detectable levels in PBMCs. Moreover, breakthrough infections always occurred in animals with high residual TFV-DP levels in PBMCs. Thus, although it is not possible to ascertain the exact contribution to the observed protection of the week-old PrEP drug levels versus those afforded by PEP application, these results suggest that under postexposure conditions, the protection seen in our study mainly reflects protection with PEP rather than residual PrEP activity from the previous dose. This does not mean the PrEP application of TAF/EVG inserts is not effective, as our previous data

show very high protection afforded by TAF/EVG PrEP when inserts were applied 4 hours before viral challenge [10]. Those studies, however, did not address the window of PrEP protection with TAF/EVG inserts.

In summary, we document an extended window of PEP protection by TAF/EVG inserts. Our observations support the clinical development of TAF/EVG inserts for flexible on-demand protection against HIV in women and inform their dosing and clinical effectiveness trial design.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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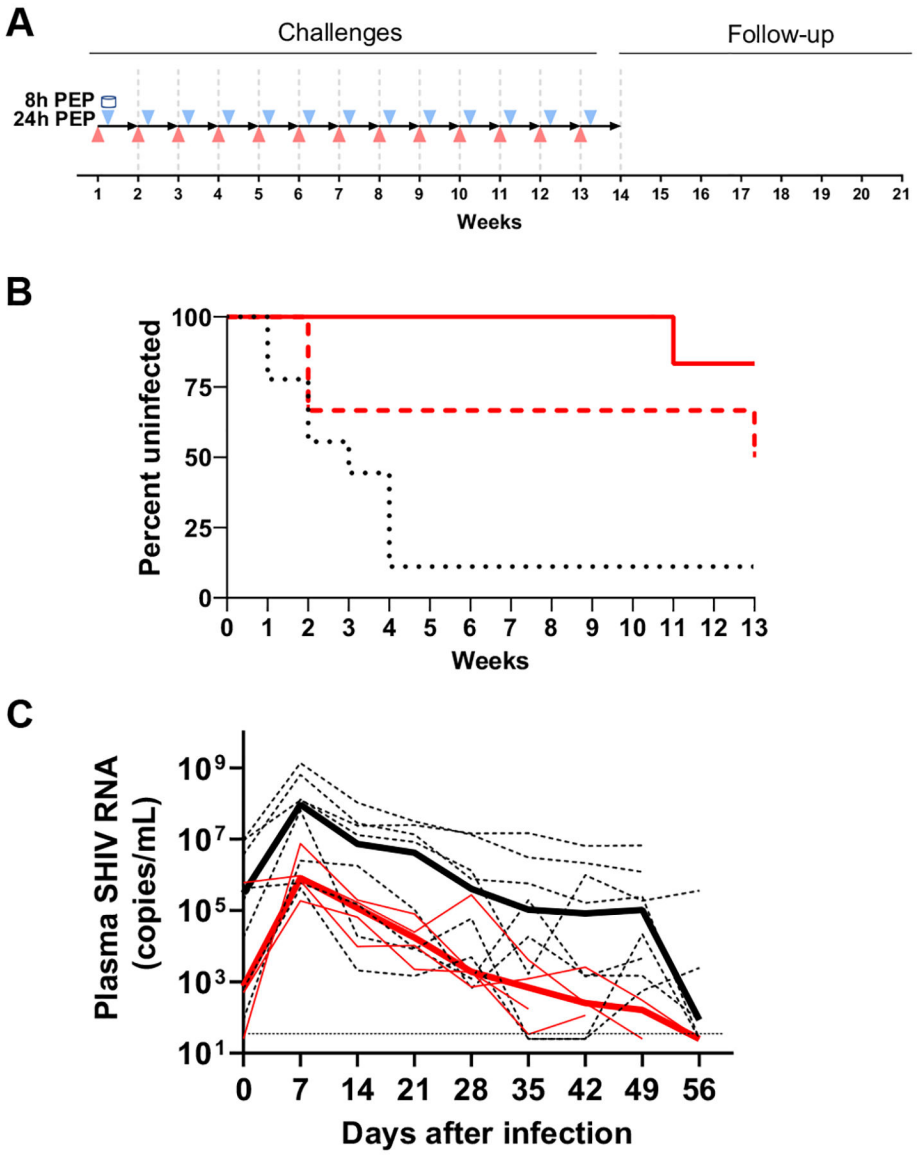

Figure 1.

Vaginal efficacy of tenofovir alafenamide fumarate/elvitegravir (TAF/EVG) inserts administered 8 or 24 h after simian/human immunodeficiency virus (SHIV) exposure. *A*, Schematic representation of studies. Red and inverted blue triangles represent virus challenges and insert applications, respectively. One TAF/EVG insert was applied 8 or 24 h after vaginal exposure with low-dose SHIV162p3 inoculum. *B*, Kaplan–Meier survival plot. The survival plot shows the cumulative percentage of uninfected macaques as a function of the number of weekly vaginal SHIV162p3 exposures. The dotted black line represents cumulative placebo controls (n = 9). The red solid and dashed lines represent TAF/EVG insert (n = 6) applied 8 or 24 h after virus exposure, respectively. Placebo controls were infected after a median of 3 exposures. At 8 and 24 h postexposure prophylaxis (PEP), the calculated efficacy was 94.41% (95% exact confidence interval [CI], 57.03%–99.27%) and 77.23% (95% exact CI, 20.00%–93.52%), respectively. Animals in both arms were

followed for an additional 8 weeks to monitor for late infections. *C*, Plasma viral load of SHIV-infected animals. Individual infected animals from real-time and historical placebo controls (n = 8, dotted black lines), and 8 h (n = 1) and 24 h (n = 3) TAF/EVG inserts (red lines) aligned to the first day when viral RNA was detected (day 0). The median for all placebo and treated animals is shown with bolded black and red lines, respectively. Values below the limit of quantification (LOQ) were given a value of one-half the LOQ (LOQ = 50 copies/mL).

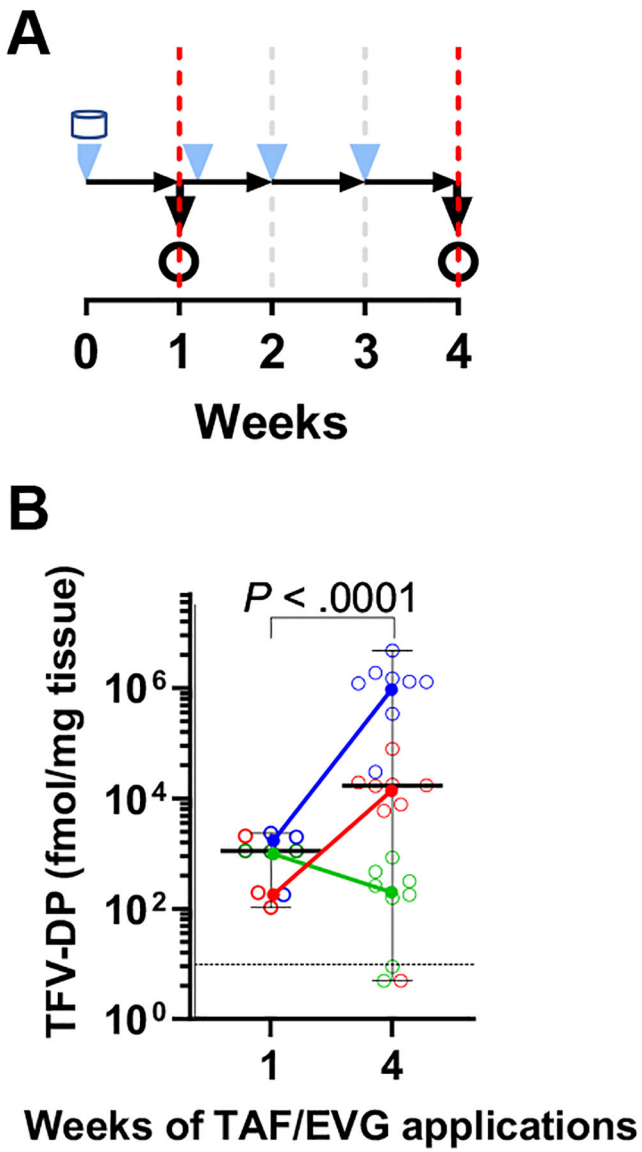

Figure 2.

The impact of repeated vaginal dosing on tenofovir-diphosphate (TFV-DP) levels at the time of virus exposure. *A*, Schematic representation of terminal pharmacokinetic study. Blue triangles and black arrows with circles represent the insert application and the time of the biopsy collection (7 d after the insert application), respectively. The second week's insert application took place 24 h after biopsy collection. *B*, Levels in individual biopsies collected from 3 animals (open circles) were plotted. Filled circles represented the mean for each animal, and the lines depict the difference between 1 tenofovir alafenamide fumarate/elvitegravir (TAF/EVG) dose and 4 weekly doses. The animals are color-coded: FJ90 is green, A12W026 is blue, and Z14276 is red. Medians of 3 animals for 1 and 4 doses are depicted with horizontal black lines. Values below the limit of quantification (LOQ; black dotted line) were given a value of one-half the LOQ (LOQ was 10 fmol/mg of tissue).