The effect of depot medroxyprogesterone acetate on tenofovir alafenamide in rhesus macaques

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Abstract

Prevention of HIV infection and unintended pregnancies are public health priorities. In sub-Saharan Africa, where HIV prevalence is highest, depot medroxyprogesterone acetate (DMPA) is widely used as contraception. Therefore, understanding potential interactions between DMPA and antiretrovirals is critical. Here, we use a macaque model to investigate the effect of DMPA on the pharmacology of the antiretroviral tenofovir alafenamide (TAF).

Female rhesus macaques received 30 mg of DMPA (n = 9) or were untreated (n = 9). Macaques received a human equivalent dose of TAF (1.5 mg/kg) orally by gavage. Tenofovir (TFV) and TFV-diphosphate (TFV-DP) were measured in blood, secretions, and tissues over 72 h. The median area under the curve (AUC_{0-72h}) values for TFV-DP in peripheral blood mononuclear cells were similar in DMPA-treated (6991 fmol*h/10^6 cells) and untreated controls (5256 fmol*h/10^6 cells) (P = 0.174). Rectal tissue TFV-DP concentrations from DMPA+ animals [median: 20.23 fmol/mg of tissue (range: 4.94–107.95)] were higher than the DMPA− group [median: below the limit of quantification (BLOQ-11.92)], (P = 0.019). TFV-DP was not detectable in vaginal tissue from either group.

A high-dose DMPA treatment in macaques was associated with increased rectal TFV-DP levels, indicating a potential tissue-specific drug-drug interaction. The lack of detectable TFV-DP in the vaginal tissue warrants further investigation of PrEP efficacy with single-agent TAF products. DMPA did not affect systemic TAF metabolism, with similar PBMC TFV-DP in both groups, suggesting that DMPA use should not alter the antiviral activity of TAF.

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Keywords

Hormonal contraception; Antiretrovirals; Macaque model; Pre-exposure prophylaxis; HIV

1. Introduction

Prevention of HIV infection and unintended pregnancies are public health priorities. Depot medroxyprogesterone acetate (DMPA) is a widely used progestin injectable contraceptive, especially in sub-Saharan Africa where HIV incidence is highest (Bertrand et al., 2014; Centers for Disease Control and Prevention; Tsui et al., 2017). A single injection provides contraception for 3 months, but does not protect against sexually transmitted diseases such as HIV (Centers for Disease Control and Prevention: U.S, 2016). Initial reports from observational studies suggested an increased risk of HIV acquisition in women using DMPA (reviewed in (Polis et al., 2016)). In 2016, the World Health Organization (WHO) made a data-driven update to the 'Medical eligibility criteria for contraceptive use' and reclassified DMPA as Category-2 for women at high risk of HIV, indicating its use outweighs the proven or theoretical risks (World Health Organization, 2015). In 2019, the Evidence for Contraceptive Options and HIV Outcomes (ECHO) clinical trial found that DMPA did not substantially affect rates of HIV acquisition compared to another hormonal contraceptive (levonorgestrel) or a nonhormonal contraceptive (copper IUD) (Evidence for Contraceptive Options and HIV Outcomes ECHO Trial Consortium, 2019). Subsequently, the WHO reverted DMPA to Category-1, indicating no restrictions for use of this contraceptive method (Evidence for Contraceptive Options and HIV Outcomes ECHO Trial Consortium, 2019; World Health Organization, 2019).

Nonhuman primate models have provided valuable insight for how hormones and hormonal contraceptives may influence HIV acquisition (reviewed in (McNicholl et al., 2014)). Early macaque studies demonstrated a correlation between endogenous hormonal fluctuations and SIV susceptibility, with the highest infection rate during the progesterone-dominated luteal or late luteal phase of the menstrual cycle (Kersh et al., 2014; Vishwanathan et al., 2010; Sodora et al., 1998; Vishwanathan et al., 2011). Exogenous hormonal treatments with progesterone, estrogen, and DMPA also modulate infection efficiency (Sodora et al., 1998; Vishwanathan et al., 2011; Smith et al., 2000; Marx et al., 1996). Specifically, a high 30 mg dose of DMPA administered intramuscularly increased the risk of vaginal SIV acquisition in rhesus macaques and accelerated progression to simian AIDS (Marx et al., 1996; Trunova et al., 2006). The biological mechanisms for increased infection risk may include thinning of the vaginal epithelium, changes in volume and chemical composition of vaginal secretions, and an altered immune response (Hild-Petito et al., 1998; Chandra et al., 2013; Mauck et al., 1999; Carias et al., 2016). Concerns regarding the translational relevance of the high dose DMPA macaque model led to defining a human equivalent dose of DMPA that better recapitulates medroxyprogesterone acetate (MPA) levels in plasma and vaginal epithelial thinning seen in women (Radzio et al., 2014a). After treatment with 1.5 mg/kg of DMPA, macaques were twice as likely to acquire vaginal SHIV, but the results were not statistically significant (Butler et al., 2016).

Macaque models also provide a comprehensive platform to analyze safety, pharmacology, and efficacy of various pre-exposure prophylaxis (PrEP) modalities (reviewed in (García-Lerma and Heneine, 2012)). Daily oral PrEP with emtricitabine (FTC) and tenofovir disoproxil fumarate (TDF) reduces the risk of HIV infection from sexual transmission in men, women and transgender women, as well as people who inject drugs (Grant et al., 2010; Thigpen et al., 2012; Baeten et al., 2012; Choopanya et al., 2013). The large-scale clinical trials that led to the approval of daily PrEP with FTC/TDF were guided by many preclinical macaque studies (Dobard et al., 2012; García-Lerma et al., 2008, 2010; Parikh et al., 2009; Subbarao et al., 2006). Furthermore, PrEP was evaluated in DMPA-treated macaques and there was no impact on the prophylactic efficacy of FTC/TDF in a SHIV vaginal challenge model (Radzio et al., 2014b). A sub-analysis of clinical trial data also showed that PrEP was efficacious for HIV-1 prevention in women using DMPA (Heffron et al., 2014). However, important pharmacological questions, such as potential drug-drug interactions during combined use of PrEP and DMPA, are being addressed in the ongoing DynamoPrEP clinical trial (NCT03197961).

Tenofovir alafenamide (TAF), a prodrug of tenofovir (TFV), has reduced renal and bone toxicity, in part, because it yields 90% less systemic TFV than TDF (Ray et al., 2016). Moreover, TAF has greater accumulation of TFV-diphosphate (TFV-DP), the active intracellular metabolite, in peripheral blood mononuclear cells (PBMCs) and lymphoid tissue than TDF (Sax et al., 2015; Arribas et al., 2017; Spinner et al., 2019; Fletcher et al., 2019). The combination of oral TAF/FTC was approved in 2019 as PrEP for men who have sex with men, but it is still unknown if TAF/FTC will be equally effective in women.

Studies showing limited penetration of TAF in female genital tissue have raised questions about the ability of TAF to prevent vaginal infection (Massud et al., 2019; Garrett et al., 2016; Cottrell et al., 2017, 2019; Nicol et al., 2020a). In a recent pig-tailed macaque study, the combination of TAF and FTC conferred 91% protection against vaginal SHIV infection while TAF alone was associated with a moderate protective efficacy of 58–73%. TFV-DP was undetectable in four out of nine vaginal biopsies in the TAF-only group, suggesting that low vaginal TFV-DP may not be adequate for protection when systemic TFV-DP concentrations are not sufficiently high (Massud et al., 2019). The pharmacological interactions between TAF and hormonal contraceptives have not been studied *in vivo*. In a recent *ex vivo* study, DMPA was found to suppress TAF metabolism in female genital tract cells although little or no effect was seen in blood cells (Shen et al., 2017). Recent clinical evaluations of TFV-based regimens also indicated that mucosal tissue TFV-DP may be affected by exogenous hormonal treatments, but not by endogenous hormonal cycles (Cottrell et al., 2019; Nicol et al., 2020a, 2020b).

Elucidating interactions between hormonal contraception and antiretrovirals are important for designing multipurpose prevention strategies that can prevent HIV and unintended pregnancies. Here, we used a rhesus macaque model to evaluate the effect of high-dose DMPA treatment (30 mg) on the metabolism of oral TAF.

2. Materials and methods

2.1. Animal care guidelines

All animal protocols were approved by the Centers for Disease Control and Prevention (CDC) Institutional Animal Care and Use Committee (IACUC). Female rhesus macaques (*Macaca mulatta*) were cared for by CDC veterinarians in compliance with the Guide for the Care and Use of Laboratory Animals 8th Ed. All procedures were performed under anesthesia (10 mg/kg ketamine or 2–6 mg/kg telazol; intramuscular). Every effort was made to minimize distress through enrichment opportunities and humane interactions. Animals were fed a commercial diet specifically formulated to meet vitamin C requirements and had access to water at all times. Compatible macaques were pair housed and single housed animals had cage dividers that, at minimum, allowed eye contact.

2.2. Study design

Fig. 1 illustrates the study, which was done in two phases. In Phase 1, four female rhesus macaques (median age 5 years [range 5–9 years]) received a human-equivalent dose of TAF (Laurus Labs) orally by gavage based on body weight (1.5 mg/kg) (Massud et al., 2016). Blood, rectal swabs, and vaginal swabs were collected at 1, 5, 24, and 72 h after TAF administration. Pinch biopsies were collected from the vagina and rectum at 24 h post TAF treatment. No further treatment was administered for 2 weeks to ensure washout of TAF and TAF metabolites. Next, intramuscular injections of DMPA (30 mg) were given to the same animals. After 11 days, sufficient time for MPA levels to peak in plasma (Radzio et al., 2014a), they received oral TAF and samples were collected as outlined above. TFV-DP data from PBMCs acquired from this phase was excluded from the study as the analysis of endogenous deoxyadenosine triphosphate (dATP) levels indicated sample degradation (data not shown).

In Phase 2, ten rhesus macaques (median age 8.5 years [range 5–13]) were untreated (DMPA-, n = 5) or received DMPA (DMPA+, n = 5) prior to being administered oral TAF (1.5 mg/kg). Blood was collected at 1, 5, 24 and 72 h, and rectal biopsies were collected at 24 h post TAF administration. Vaginal biopsies and secretions were not collected during Phase 2 due to the low/undetectable results from Phase 1.

2.3. Blood, tissue, and swab processing

Blood was collected in BD Vacutainer® CPT™ Cell Preparation Tubes and PBMCs were isolated following the manufacturer's protocol. Plasma was immediately frozen at − 70 °C in 500 µl aliquots for TFV, medroxyprogesterone acetate (MPA), and progesterone analysis. PBMCs were treated with BioLegend red blood cell (RBC) lysis buffer to minimize interference of RBCs in TFV-DP quantification. Cells counts and viability were determined using trypan blue exclusion on the Countess I (Invitrogen).

Rectal and vaginal tissues biopsies were collected using medical forceps with three pinches collected at each site. Extraneous liquid was carefully removed, and tissue was weighed on an analytical balance; with a median of 15.9 mg of rectal tissue and 20.3 mg of vaginal tissue collected per sample. Due to the small amount of tissue collected from four animals

in the DMPA− group in Phase 1, tissues were combined into two pools (two animals each) to ensure sufficient yield of TFV-DP and dATP. This resulted in only two data points for analysis (denoted by triangles in Fig. 3A and B). Biopsies were not combined in any of the subsequent experiments from Phase 1 or 2.

Swabs (Fisherbrand™ Synthetic-tipped Applicator) were inserted in the vagina and rectum for 5 min to ensure uptake of mucosal secretions. The volume of fluid for each sample was determined by weighing the swabs pre- and post-collection.

2.4. Measurement of intracellular TFV-DP and dATP from blood and tissue

Following an established protocol, PBMCs and tissue biopsies were resuspended in 500 μl ice-cold 80% methanol, vortexed for 1 min and immediately frozen at − 70 °C. At the end of the study, all samples were pelleted to remove cellular debris and supernatants were transferred to new tubes. Supernatants were dried, reconstituted in 100 μl of 50 mM ammonium acetate buffer (pH 7.0), and centrifuged at $17,000 \times g$. Intracellular TFV-DP and dATP concentrations were measured using an automated on-line weak anion-exchange solid-phase extraction method coupled with ion-pair chromatography-tandem mass spectrometry. Calibration curves were generated from standards of TFV-DP and dATP by serial dilutions in 80% methanol ranging from 0.25 to 10 nM. The lower limit of quantification (LOQ) is 100 fmol/sample, or in our case, 25 fmols/10^6 PBMCs because approximately 4×10^6 cells were used for extraction. All calibration curves had r^2 values of greater than 0.99 (Kuklenyik et al., 2009a; García-Lerma et al., 2011). Values below the LOQ (BLOQ) were assigned an arbitrary value of 12.5 fmol TFV-DP/10^6 cells or half of the LOQ.

2.5. Measurement of TFV in plasma and mucosal fluids

As described elsewhere, TFV in plasma and mucosal secretions was measured by high-performance liquid chromatography–tandem mass spectrometry (LC-MS/MS). TFV concentrations were calculated from a standard curve with a range of 0.5–2000 ng/ml. The lower LOQ is 10 ng per ml of plasma and 1 ng per swab (García-Lerma et al., 2010; Kuklenyik et al., 2009b).

2.6. Measurement of MPA and progesterone

MPA and progesterone were measured by Wisconsin National Primate Center Assay Services. Briefly, plasma, standards and quality control samples were diluted in 500 μL of ultrapurified water (Fisher Scientific). Methyl tert butyl ether (Fisher Scientific) was then added, vortexed vigorously, and incubated at room temperature for 5 min. The organic phase containing steroids was transferred into a new tube, evaporated to dryness by air stream and heated water bath (60 °C), and then resuspended in 50 μL of 20% acetonitrile in water and analyzed via LC-MS/MS. The calibration curve ranged from 0.195 to 25 ng/mL and the linearity was r > 0.9990. The intraassay CV was 2.76% and the interassay CV was 4.7%.

2.7. Statistical analysis

Medians were calculated for DMPA-treated and untreated groups for seven outcomes (PBMC TFV-DP area under the curve $(AUC)_{0-72h}$, PBMC TFV-DP maximum concentration (C_{max}), PBMC dATP/TFV-DP ratio, rectal biopsy TFV-DP, rectal secretion TFV, and vaginal

secretion TFV). Differences in medians between the DMPA-treated and untreated groups were evaluated using the Wilcoxon rank sum test. For a visual inspection, median TFV-DP concentrations and dATP/TFV-DP ratios in macaque PBMCs from 1 to 72 h were graphed. Concentrations of MPA and progesterone in plasma from day 11–14 post DMPA treatment (30 mg, intramuscular) were also graphed. Figures were made using GraphPad Prism version 8.41, GraphPad Software (La Jolla California, USA).

3. Results

3.1. Study schematic

We used a rhesus macaque model to evaluate potential interactions between DMPA and TAF. As shown in Fig. 1, female macaques received no hormonal treatment (blue, DMPA-) or a 30 mg dose of DMPA intramuscularly (red, DMPA+). To ensure sufficient time for the systemic distribution of MPA and functional effects, e.g. suppression of progesterone production and thinning of the vaginal epithelium (Radzio et al., 2014a), we delayed TAF treatment by 11 days after DMPA administration. Next, macaques were given a human equivalent dose of TAF (1.5 mg/kg) orally by gavage and blood, mucosal secretions, and mucosal biopsies were collected over 72 h.

3.2. DMPA does not affect systemic TAF exposure

Plasma was analyzed for extracellular TFV. As expected, median plasma TFV levels were low, with detectable TFV in only 3 of the 18 samples, all at the 1-h timepoint (data not shown). One sample was from the DMPA− group (39 ng/ml) and the other two were from the DMPA + group (15 and 19 ng/ml).

Next, we evaluated whether DMPA alters the concentrations of TFV-DP in PBMCs. Fig. 2A illustrates the levels of TFV-DP in PBMCs at 1, 5, 24, and 72 h after TAF administration. The median PBMC TFV-DP $AUC_{0–72h}$ in the DMPA+ group (6991 fmol*h/10^6 cells [range: 4708–18,083]), was higher than the DMPA− group (5256 fmol*h/10^6 cells [range: 2318–9, 958]), but this difference was not statistically significant (P = 0.174). The median C_{max} of TFV-DP in PBMCs was higher in the DMPA+ compared to the DMPA− group; 154.5 fmol/10^6 cells [range 132.7–308.1] and 105.9 fmol/10^6 cells [range 40.0–200.8]. Interestingly, this difference was statistically significant (P = 0.047) (Table 1).

We also measured the concentrations of endogenous dATP since it competes with TFV-DP for incorporation by HIV reverse transcriptase (RT) during viral genomic replication. The antiviral activity TFV-DP may be reduced by increased dATP levels, as the natural nucleotide limits RT incorporation of TFV-DP into nascent cDNA, which subsequently induces chain termination (García-Lerma et al., 2011; Arts et al., 1996). Fig. 2B shows dATP/TFV-DP ratios for each animal at every time point after TAF administration. Median dATP/TFV-DP ratios were similar in DMPA− and DMPA+ animals (9.1 [range: 2.8–65.2] and 7.3 [range: 2.9–67.6], respectively, P = 0.482). Taken together, these data suggest little or no effect of DMPA on the antiviral activity of TFV-DP in PBMCs.

3.3. Limited drug exposure in vaginal tissue

Vaginal biopsies were collected 24 h post TAF administration from four DMPA-treated and untreated animals. Fig. 3A shows that TFV-DP was undetectable in all vaginal samples, whether from a single animal or combined tissue from two animals (Fig. 3A). This data is consistent with extremely low or undetectable levels seen in other macaque and human studies (Massud et al., 2019; Garrett et al., 2016; Cottrell et al., 2017). Efficient nucleotide extraction from vaginal tissue was confirmed by measuring dATP levels. DMPA had no observable effect on dATP concentrations in vaginal biopsies. Table 1 shows that very low levels of TFV was detected in vaginal secretions with median C_{max} values of 1.34 ng/ml [range: 0.04–2.88] and below the limit of quantification (BLOQ) [range: BLOQ - 7.60] in the DMPA− and DMPA + groups, respectively (P = 0.237). Taken together, these data suggest limited TAF penetration in the vaginal compartment.

3.4. DMPA was associated with increased rectal TFV-DP

Rectal biopsies were collected 24 h after TAF administration from nine animals in the absence or presence of DMPA treatment. As described in the methods, four animals from the DMPA− group had very limited tissue collected, and biopsies from these animals were thus combined into two pools. This gave a total of seven measurements in the DMPA− group; five from individual animals and two from the combined biopsies. TFV-DP was BLOD in five out of seven samples (Fig. 3B). The two samples with detectable TFV-DP were from the combined rectal tissues suggesting tissue input may be an important factor for assessing TFV-DP from rectal biopsies. Interestingly, rectal tissue TFV-DP concentrations from DMPA+ animals [median: 20.23 fmol/mg of tissue (range: 4.94–107.95)] were higher than the DMPA− group [BLOQ (BLOQ-11.92)], (P = 0.019). The increased TFV-DP observed in DMPA-treated animals did not correlate with an increase in dATP extracted from the same tissue (Fig. 3B). Despite the higher TFV-DP in rectal tissue, TFV in rectal secretions were not different with a C_{max} of 25.64 ng/ml [range: 1.51–53.09] and 31.56 [range: 5.65–41.21] in the DMPA-and DMPA+ groups, respectively (P = 0.564) (Table 1).

3.5. MPA and progesterone in plasma

We measured MPA levels in plasma 11–14 days after DMPA administration (Fig. 4A). As expected, no MPA was detected in the untreated animals. Median MPA levels in the DMPA+ animals were 7.33 ng/ml [range: 2.15–39.14] and consistent with previously published results (Radzio et al., 2014a). We observed similar endogenous plasma progesterone concentrations to prior studies in pig-tailed macaques (Kersh et al., 2014; Butler et al., 2016), and as anticipated, DMPA+ animals had suppressed plasma progesterone, likely due to MPA inhibition of ovulation which has been observed in macaques and humans (Fig. 4B) (Kersh et al., 2014; Butler et al., 2016; Jain et al., 2004).

4. Discussion

The present study evaluated the effect of DMPA on the pharmacology of TAF in rhesus macaques. We found that DMPA did not affect the systemic metabolism of TAF and that PBMC TFV-DP AUC_{0-72h} values were similar in DMPA-treated and untreated animals. Despite a higher TFV-DP C_{max} in DMPA-treated animals, the similar dATP/TFV-DP ratios

in PBMCs from both groups indicate little or no effect of DMPA on the predicted antiviral activity of TAF. These findings are reassuring and suggest that concomitant use of DMPA and regimens containing TAF will not affect virologic outcomes.

HIV treatment has well defined thresholds for systemic concentrations of antiretrovirals required to maintain viral suppression. Identifying protective benchmarks for novel prophylaxis agents is complex, in part, due to the broad range of transmission routes (e.g. vaginal, rectal, perinatal, etc.), the distinct pharmacology of each antiretroviral and their subsequent formulations (Abdool Karim et al., 2011). Daily oral PrEP (FTC/TDF) is highly effective for HIV prevention in men who have sex with men, heterosexual men and women. Clinical trials have highlighted adherence to medication as one of the strongest correlates of protection (reviewed (Chou et al., 2019)). In 2012, The Partners PrEP trial evaluated oral TDF/FTC PrEP in serodiscordant heterosexual couples and showed 66% and 84% protective efficacy in women and men, respectively, a difference that was not statistically significant (Baeten et al., 2012; Murnane et al., 2013). The most recent update from the Partners PrEP trial showed 93% efficacy of oral TDF/FTC in 2014 (Baeten et al., 2014). Similarly, the Botswana TDF2 trial showed 78% protective efficacy of TDF/FTC in serodiscordant heterosexual couples, although this study was not powered to discriminate between sex (Thigpen et al., 2012). The VOICE and FEM-PrEP clinical trials assessed oral TDF/FTC in high risk heterosexual women and both were terminated early due to no reductions in the rate of HIV acquisition, which was largely attributed to low medication adherence as determined by plasma TFV (Marrazzo et al., 2015; Van Damme et al., 2012). Although, adherence is a definitive factor for PrEP efficacy, there may be other sex-specific factors, such as hormonal fluctuations, that play a role for protection in women.

Studies in women have shown lower penetration of TAF in the female genital tract compared to TDF. After TAF treatment, TFV-DP levels in vaginal tissues were BLOQ in 89–95% of samples tested compared to about half of the samples from women treated with TDF (Garrett et al., 2016; Cottrell et al., 2017). In pigtailed macaques treated with a human-equivalent dose of TAF, TFV-DP levels achieved with TAF were also undetectable in 45% of vaginal biopsies (Massud et al., 2019). Here, we show no detection of TFV-DP in vaginal tissues from rhesus macaques receiving the same TAF dose, further confirming the reduced efficiency of TAF to load vaginal tissue with TFV-DP relative to TDF. However, despite this low tissue penetration, single-agent TAF conferred 58%–73% protection against vaginal SHIV challenge, highlighting an important role for TFV-DP in PBMCs for TAF as PrEP (Massud et al., 2019).

Our experimental approach had some limitations that may have reduced our ability to accurately measure TFV-DP in vaginal tissues. Vaginal pinch biopsies generally yield small amounts of tissue, which for this study ranged from 13 to 46 mg. It is possible that increasing the amount of tissue might improve TFV-DP detection. We found that TFV-DP can be consistently detected in vaginal tissue collected at necropsy when biopsy size is not limited (unpublished data). Therefore, larger biopsies or combining biopsies from multiple animals may be needed to accurately assess TFV-DP in rhesus macaque vaginal tissue. Also, sampling at multiple time points may be beneficial as Cottrell et al. found that female genital tract TFV-DP peaked at 6 h and with the latest quantifiable levels at 24 h (Cottrell et al.,

2017). Another limitation of our study was the use of a high dose of DMPA. We selected the 30 mg dose since this dose is commonly used to ensure vaginal infection of rhesus macaques with SIV and SHIV and to assess biomedical interventions to prevent infection (reviewed in (Veazey et al., 2012)). It was reassuring to see that the PK profile of TAF in blood was not affected despite the use of this high DMPA dose. These results are in line with our previous observations showing no effect of a lower 4 mg DMPA dose on the PK profile of FTC and TDF (Radzio et al., 2014b).

Although sexual transmission of HIV in women is most often associated with vaginal intercourse, the prevalence of heterosexual anal sex is high and contributes substantially to new infections (Hess et al., 2016; Elmes et al., 2020). Importantly, heterosexual rectal intercourse is associated with a higher risk of HIV acquisition (Baggaley et al., 2010). To ensure broad protection, prophylactic assessment of antiretrovirals for women must consider the extent of drug penetration in the rectal compartment. Cottrell et al. simulated TFV-DP levels in rectal tissue with daily TDF dosing and estimated 100% protection, however, TAF dosing yielded 13-fold lower TFV-DP in rectal tissue with 63% of samples below the LOQ (Garrett et al., 2016). Here, we observed a similar trend towards lower TFV-DP levels in rectal tissues with TAF compared to TDF. In male rhesus macaques treated with TAF (1.5 mg/kg), TFV-DP was detected rectal biopsies in three out of four animals with a median of 6.6 (range BLOQ-15.4) fmol TFV-DP per mg of tissue (Massud et al., 2016), which was higher than the DMPA-untreated females and lower than the DMPA-treated females, (medians = BLOQ and 20.23 fmol/mg tissue, respectively). This sex-specific discrepancy in TFV-DP is not observed in PBMCs, with similar levels in males and females with or without DMPA treatment (Massud et al., 2016).

In our model the effects of DMPA on systemic TAF exposure are predicted to be negligible, however we observed a statistically significant increase of TFV-DP in rectal tissue. Drug-drug interactions can occur due to overlapping or interacting metabolic pathways. We considered the enzymes responsible for the intracellular conversion of TAF to TFV, cathepsin A and carboxylesterase, but there are no publications indicating DMPA alters these enzymes. DMPA is a substrate for and inducer of cytochrome P450 3A4 (CYP3A), a major component in drug metabolism, however TAF is not a substrate for CYP3A (Gilead, 2015). Next, we considered the tissue-specificity of our results. Compartment specific effects between MPA and TAF have been observed *in vitro*, where MPA treatment led to a reduction in female genital tract cell TFV-DP, with no effect on blood cells (Shen et al., 2017). $5^{'}$ nucleotidases, a class of enzymes responsible for maintaining physiological nucleotide balance, are altered by hormone exposure in the female genital tract (Bucci and Murphy, 2001). To our knowledge, the impact of hormones on 5' nucleotidases in rectal tissue is unknown. Lastly, a sub-analysis of transgender women using feminizing hormone therapy (FHT) from the iPrEX trial showed significantly increased dATP concentrations in rectal tissue compared to cisgender men or women that were not on contraceptives. This study also found higher median TFV-DP in rectal tissue in transgender men and cisgender women compared to cisgender men, although this was not statistically significant (Cottrell et al., 2019). There is not a clear biological mechanism but taken together these data indicate hormonal treatments may impact nucleotide regulation in tissue.

In summary, we show that DMPA does not affect the systemic exposure of TAF in plasma and does not alter the predicted efficacy of TFV-DP in PBMCs. However, we noted an increase in rectal accumulation of TFV-DP. Although limited by the small sample size and the high dose of DMPA used, our results are reassuring as they suggest that the injectable contraceptive DMPA may not affect the pharmacological profile and antiviral activity of TAF.

Acknowledgments

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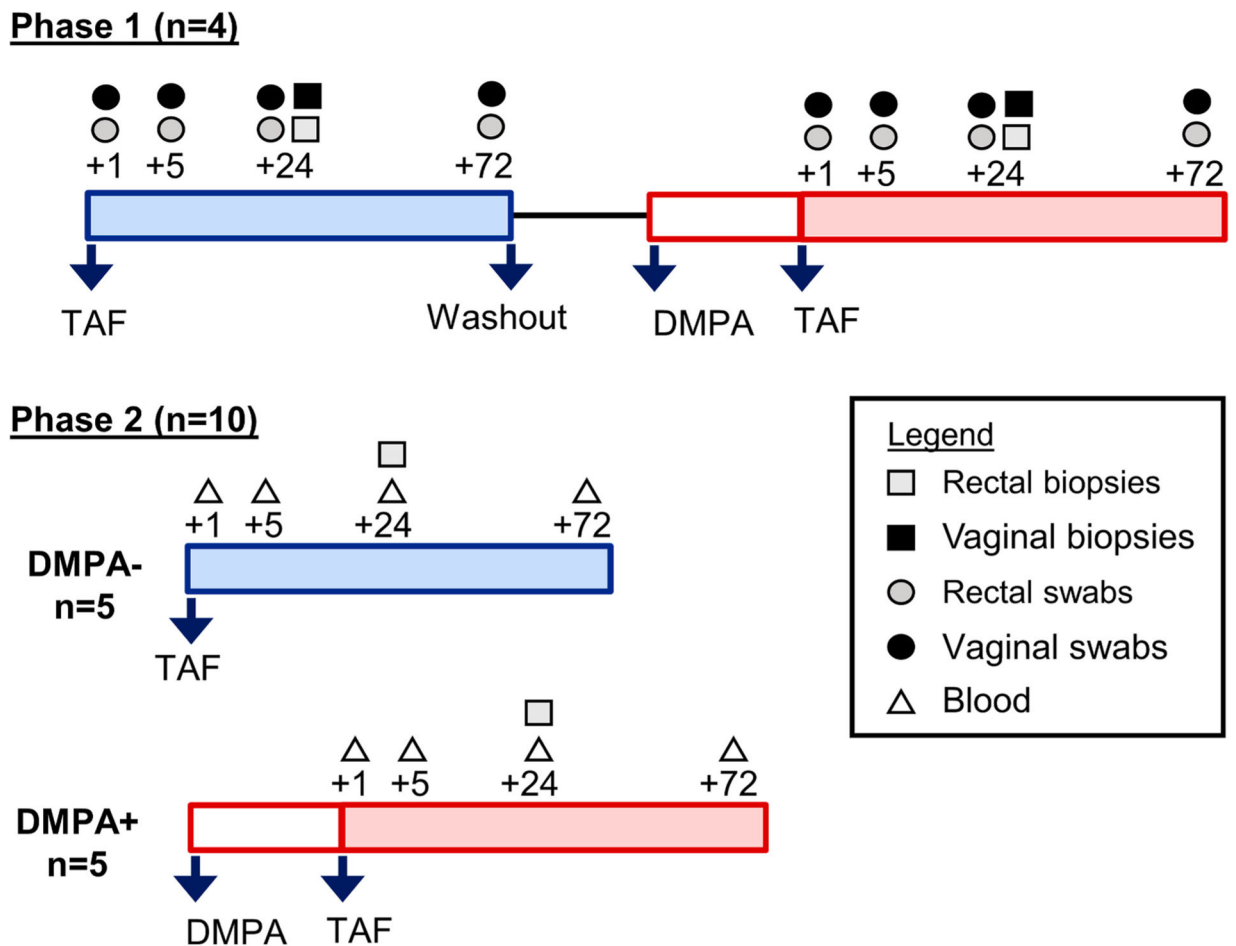
Fig. 1. Study design.

In Phase 1, Female macaques (n=4) were administered oral TAF (1.5 mg/kg). Mucosal biopsies and secretions were collected at the indicated time points. After a two-week washout period, the same animals were treated with DMPA and TAF, and samples were collected. In Phase 2, macaques were either untreated (DMPA−, n=5) or received DMPA (DMPA+, n=5). Both groups received oral TAF, and blood and rectal biopsies were collected.
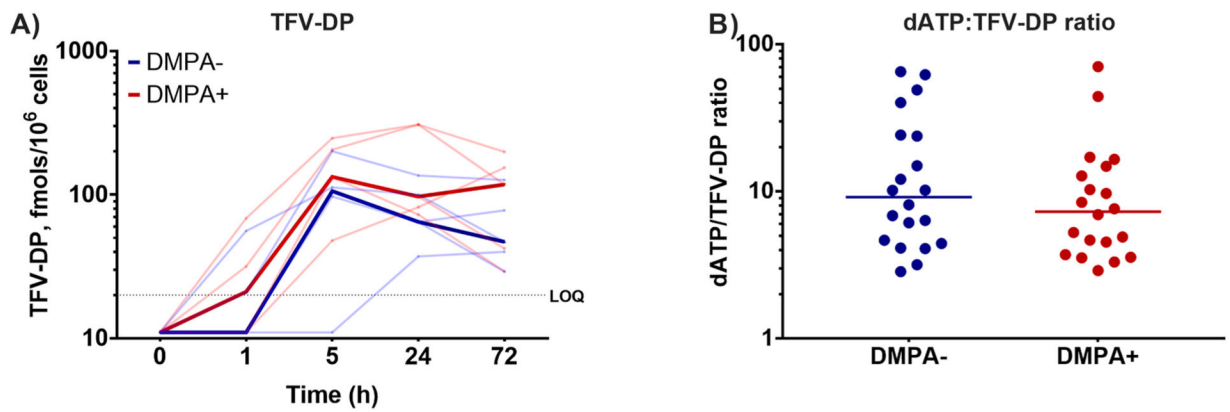
Fig. 2. Intracellular TFV-DP concentrations and dATP/TFV-DP ratios in macaque PBMCs.
(**A**) Median TFV-DP concentrations in macaque peripheral blood mononuclear cells (PBMCs) are shown over time. Medians are illustrated by solid lines and faint lines represent individual animal data from the untreated (n=5, blue) or DMPA-treated macaques (n=5, red). (**B**) The median dATP/TFV-DP ratio is indicated by colored bar, and circles indicate dATP/TFV-DP at every time point (1–72 hours) after TAF administration for all animals.
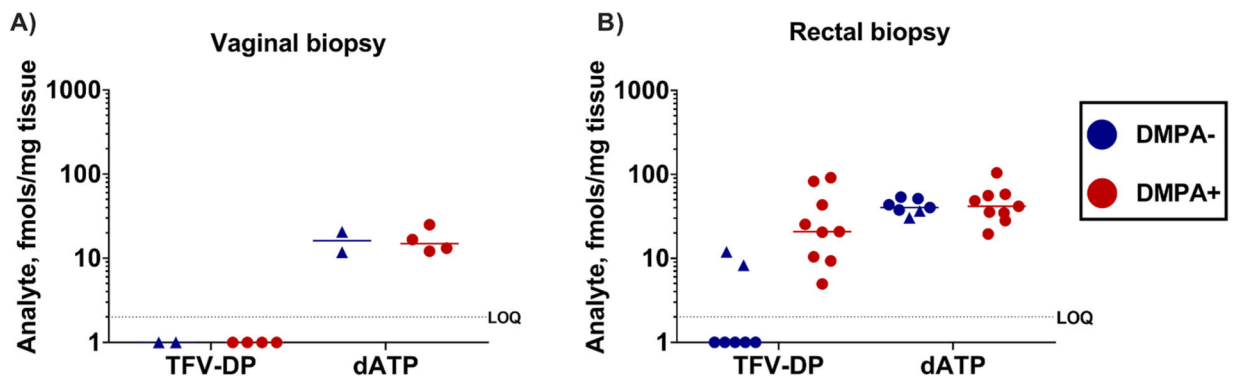
Fig. 3. Median intracellular TFV-DP mucosal tissues.
Median TFV-DP and dATP concentrations in **(A)** vaginal and **(B)** rectal tissue collected
24 hours post TAF administration. Median nucleotide concentrations are indicated by bar,
with individual data points represented by shapes. Circles represent extractions from a single
animal and triangles represent extractions from tissue combined from two animals.
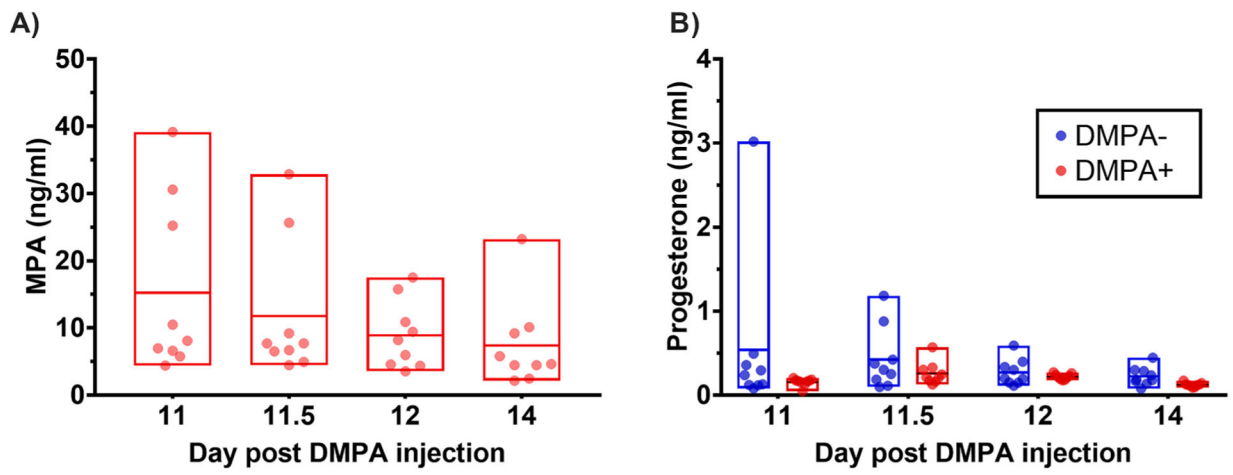
Fig. 4. Concentrations of medroxyprogesterone acetate and progesterone in plasma.
Plasma levels of medroxyprogesterone acetate (MPA) **(A)** and progesterone **(B)** were characterized from day 11–14 post DMPA treatment (30 mg, intramuscular).

Table 1.

Pharmacokinetic profile of oral TAF ± DMPA

PK Parameter: median [range]	DMPA−	DMPA+
PBMC (n=5)		
TFV-DP AUC_{0-72h} (fmol·h/10^6 cells)	5256 [2318 – 9958]	6991 [4708 –18083]
c_{max} (fmol/10^6 cells)	105.93 [40.04 – 200.83]	154.46 [132.66 – 308.11]
Rectal compartment (n=9)		
Biopsy TFV-DP T_{24h} (fmol/mg tissue)	BLOQ [BLOQ – 11.92]	20.23 [4.94 – 107.95]
Secretion TFV Cmax (ng/ml)	25.64 [1.51 – 53.09]	31.56 [5.65 – 41.21]
Vaginal Compartment (n=4)		
Biopsy TFV-DP T_{24h} (fmol/mg tissue)	BLOQ	BLOQ
Secretion TFV Cmax (ng/ml)	1.34 [0.04 – 2.88]	BLOQ [BLOQ – 7.60]

BLOQ= Below limit of quantification