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Depot-medroxyprogesterone acetate does not reduce the prophylactic efficacy of emtricitabine and tenofovir disoproxil fumarate in macaques

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Abstract

Concerns that the injectable contraceptive depot-medroxyprogesterone acetate (DMPA) may increase the risk of HIV acquisition in women led to questions on whether DMPA could reduce efficacy of pre-exposure prophylaxis (PrEP) for HIV prevention. We used a macaque model to investigate the impact of prolonged DMPA on PrEP with FTC/TDF. Twelve pigtail macaques treated with DMPA were exposed vaginally to SHIV once a week for up to 5 months and received either placebo (n=6) or FTC/TDF (n=6). All control macaques were infected while the PrEP-treated animals remained protected (p=0.0007). This model suggests that women using DMPA will fully benefit from PrEP.

Keywords

Depot-medroxyprogesterone acetate; injections; contraception; macaques; pre-exposure prophylaxis; simian HIV

Introduction

Hormonal contraceptive methods are highly effective at preventing unintended pregnancies and reducing maternal mortality and pregnancy-related morbidity, particularly in resource-limited settings. In sub-Saharan Africa where the HIV prevalence is highest, depot-medroxyprogesterone acetate (DMPA) represents the most commonly used method of contraception [1]. However, some observational studies have suggested that women using DMPA may be at higher risk for HIV infection and may be more likely to infect their HIV negative male partner [reviewed in [2, 3]]. Although observational studies may be confounded by many factors including differences in sexual behavior, other studies point to

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biological mechanisms for increased HIV risk due to DMPA including changes in vaginal structure and immune modulatory effects. In some studies, DMPA was found to thin the vaginal epithelium to luteal phase levels and increased the number of HIV target cells in vaginal tissues although other studies found no effect of DMPA on thickness or immune cell populations [4-7]. In pigtail macaques, DMPA reduces vaginal epithelium thickness and increases the percentage of $CD3^+$ cells in a dose-dependent manner [8].

Pre-exposure prophylaxis (PrEP) with tenofovir disoproxil fumarate (TDF), alone or in combination with emtricitabine (FTC) is a novel HIV prevention strategy that can reduce the risk of sexually acquired HIV infection in both men and women [reviewed in [9]]. PrEP with daily FTC/TDF has been approved in the US for HIV prevention in high-risk populations. Since women using DMPA could combine DMPA with PrEP in order to prevent HIV infection, it is important to understand if biological changes associated with DMPA may potentially reduce the prophylactic efficacy of FTC/TDF. We recently developed a pigtail macaque model of DMPA that recapitulates many of the biological effects of DMPA seen in women. This model uses a low DMPA dose that is sufficient to suppresses ovulation while achieving reductions in vaginal epithelium thickness that are moderate in magnitude [8]. The DMPA dose was defined through careful DMPA titration and analysis of several biologic and pharmacokinetic parameters including plasma MPA concentrations and changes in the vaginal epithelium. Here, we integrated this new DMPA model with a repeat low-dose vaginal SHIV transmission model that demonstrated efficacy of FTC/TDF in normally cycling pigtail macaques [10].

Materials and Methods

Drugs and viruses

TDF and FTC were kindly provided by Gilead Sciences and were given orally by gavage based on body weight as a single solution containing 20 mg/kg FTC and 22 mg/kg TDF [10]. Macaques were anesthetized using standard doses of ketamine hydrochloride. The $SHIV_{162p3}$ virus challenge stock was obtained from the NIH AIDS Research repository and expanded in pigtail macaque PBMCs prior to this study [10].

Study design

The efficacy of FTC/TDF in preventing vaginal transmission was evaluated using an established pigtail macaque model consisting of repeated vaginal exposures to a low dose (50 $TCID_{50}$) of an R5-tropic $SHIV_{162p3}$ isolate [10]. The specific study design is shown in Figure 1a. SHIV exposures were performed once a week for up to 20 weeks by non-traumatic inoculation of 1 mL of virus into the vaginal vault via a sterile gastric feeding tube of adjusted length; exposures were stopped when a macaque became SHIV RNA positive. Anesthetized macaques remained recumbent for at least 15 min after each intra-vaginal inoculation. Six macaques received FTC/TDF and 6 received phosphate buffer saline (PBS). Drug or PBS was given 24h before each virus exposure followed by a second dose 2h post-exposure. The average age (13.1 and 13.7 years), weight (8.3 and 8.8 kg) and peak progesterone levels (4,420 and 3,763 ng/mL) prior to DMPA administration was similar

between the treated and placebo groups ($p>0.5$ for each comparison, Mann-Whitney two-tailed t-test).

All 12 macaques received six cycles of 3 mg DMPA starting the month prior to the first virus challenge. Each DMPA cycle was 4 weeks in length except the third cycle, which was five weeks in length because of logistics that made not possible to administer DMPA at the appropriate time. Blood samples were collected throughout the course of the study to monitor for progesterone, medroxyprogesterone acetate (MPA), and infection status. Progesterone levels were measured by liquid chromatography/mass spectrometry. MPA was quantified by a commercial radio immune assay (Immunometrics, London UK) [8].

SHIV RNA was quantified by an RT-PCR assay with a sensitivity of 50 RNA copies/ml. Proviral DNA was quantified using a double-stranded primer assay with a detection limit of 3 DNA copies per million PBMCs [10]. Virus-specific serologic responses were measured using a synthetic-peptide enzyme immunoassay [10]. Animals were considered protected if they remained seronegative and negative for SHIV plasma RNA or proviral DNA during the 20 weeks of PrEP and 16 weeks of follow up. All animal procedures were approved by the CDC Institutional Animal Care and Use Committee.

Effect of DMPA on TFV and FTC pharmacokinetics

At study completion, the 6 PrEP-protected macaques where maintained on DMPA and used to evaluate the effect of DMPA on FTC and TFV pharmacokinetics. FTC and TDF were administered by gavage as a single oral dose two weeks after DMPA injection when plasma MPA levels are highest [8]. Drug concentrations were measured at 2h, 5h, 24h and 48h in plasma and vaginal secretions by high-performance liquid chromatography-tandem mass spectrometry [8]. The six macaques underwent the same pharmacokinetic study in the absence of DMPA after a six months resting period. Peak drug concentrations and area under the curve (AUC) values in DMPA-treated and untreated macaques were compared using a two-tailed Wilcoxon rank-sum test.

Results

Plasma progesterone and MPA in macaques receiving monthly DMPA cycles

A total of 12 pigtail macaques received six cycles of 3 mg DMPA starting the month prior to the first virus challenge. Figure 1a shows the experimental design. We monitored progesterone and MPA concentrations in plasma to ensure suppression of progesterone production, and confirm that MPA levels with the 3 mg DMPA dose were within the range seen in women receiving 150 mg. Figure 1b shows that peak MPA levels in plasma (median=2.4 ng/ml; min, max=1.4-4.8 ng/ml) were similar to those seen in women (2.5 ng/ml) [11], and sufficient to fully suppress progesterone production. The concentrations of MPA and progesterone in plasma in each of the 12 macaques prior to and during administration of DMPA are shown in supplementary Figure S1. In 5 macaques (1 placebo, 4 FTC/TDF), progesterone transiently spiked coinciding with the missed DMPA dose at week 4 of the third cycle (week 9 of study). However, subsequent DMPA administration in these animals at week 10 rapidly suppressed progesterone (Figure S1).

Prophylactic efficacy of FTC/TDF in macaques treated with DMPA

All 12 macaques treated with DMPA were exposed vaginally to $SHIV_{162p3}$ once a week for up to 20 weeks. The first dose of DMPA was administered at week-4. The first SHIV RNA challenge was performed at week 1. Figure 1c shows that all 6 placebo controls were infected two each at exposures 3, 4, and 6. In contrast, all 6 macaques receiving FTC/TDF were uninfected despite receiving 14-17 more SHIV challenges than controls, reflecting a robust protection by FTC/TDF (p=0.0007, log-rank test).

Effect of DMPA on systemic and mucosal FTC and TFV concentrations

The pharmacokinetic profile of FTC and TDF was evaluated in 6 macaques during their regular menstrual cycle or following administration of DMPA. Figure 2 shows the AUC and C_{max} values for FTC and TFV in plasma and vaginal secretions. Plasma $AUC_{0\text{-}24h}$ and C_{max} and values for FTC in the DMPA-treated animals (median=12,245 ng*hr/ml and 1,488 ng/ml, respectively) were similar to those seen in DMPA-untreated macaques (median=17,804 ng*hr/ml and 2,524 ng/ml, respectively) (p=0.48 and p=0.31 for $AUC_{0\text{-}24h}$ and C_{max} comparisons). Plasma $AUC_{0\text{-}24h}$ and C_{max} values for TFV were also similar among DMPA-treated (median=4,122 ng*hr/ml and 267 ng/ml, respectively) and DMPA-untreated animals (median=5,230 ng*hr/ml and 318 ng/ml, respectively) (p=0.59 for both comparisons) (Figure 2a).

In vaginal secretions, there was a possible trend toward higher $AUC_{0\text{-}48h}$ and Cmax and values for FTC in DMPA-treated (median=668,824 ng*hr/ml and 57,889 ng/ml, respectively) than in DMPA-untreated (median=122,646 ng*hr/ml and 7,230 ng/ml and, respectively) animals (p=0.09 and p=0.06 for $AUC_{0\text{-}24h}$ and C_{max} comparisons). Likewise, $AUC_{0\text{-}48h}$ and Cmax and values for TFV in DMPA-treated (median=179,095 ng*hr/ml and 8,784 ng/ml, respectively) were 2-3 times as high as those seen in DMPA-untreated (median=51,235 ng*hr/ml and 2,577 ng/ml, respectively) although the difference was not statistically significant (p=0.39 and p=0.31) (Figure 2b). Supplementary Figure S2 shows the pharmacokinetic curves for FTC and TFV in plasma and vaginal secretions.

Discussion

Prevention of HIV infection and unintended pregnancies are both public health priorities. The wide use of DMPA for contraception in women and the new availability of PrEP with FTC/TDF for HIV prevention underscore the importance of understanding the interactions between DMPA, susceptibility to HIV-1, and PrEP efficacy [1]. Randomized controlled clinical trials can assess the impact of DMPA on HIV acquisition and PrEP efficacy, but these trials are expensive and difficult to conduct. We used a macaque model that predicted FTC/TDF efficacy in women to assess to impact of a physiologic DMPA dose on PrEP. We demonstrate that FTC/TDF maintained high protection under continuous DMPA for 5 months. The efficacy seen in our study was identical to that seen in macaques with regular menstrual cycles [10], and suggests little or no impact of DMPA on PrEP with FTC/TDF.

Our study design incorporates biologically relevant models of HIV transmission, risk, and prevention. We used pigtail macaques since they have normal lunar menstrual cycles and

fluctuations in sex hormone levels that are similar to women [12]. We modified this model by treatment with a physiologic 3 mg DMPA dose that effectively suppresses ovulation while resulting in plasma MPA exposure (AUC and C_{max}) within the range seen in women receiving 150 mg of DMPA every three months [8]. The 3 mg dose also results in moderate changes in vaginal epithelial thickness as opposed to the 30 mg dose historically used in macaques which significantly reduces epithelial thickness [8]. The vaginal challenge component used physiologic virus doses and repeated virus exposures to model high-risk human exposure. FTC and TDF were given orally at doses that mimic systemic drug concentrations in humans [10, 13]. Thus, the high protection observed in this model strongly suggests that the impact of DMPA on PrEP with FTC/TDF in women may be minimal. Since our results were seen with only 2 weekly doses of FTC/TDF they suggest that daily dosing resulting in intracellular drug accumulation will also be effective.

We further conducted pharmacokinetic studies to examine effect of DMPA on drug distribution. DMPA has been associated with increases in HIV target cells and cellular activation in vaginal mucosa in women [4, 5]. A high density of immune cells in the vaginal mucosa might conceivably require more drug coverage of susceptible target cells and alter drug protection thresholds by PrEP. Likewise, cellular activation enhances infectivity and is known to increase intracellular dNTP concentrations which may reduce the antiviral activity of FTC-TP and TFV-DP by increasing competition with the natural dCTP and dATP substrates [14, 15]. We found that DMPA does not diminish vaginal drug exposures and, in fact, observed a possible trend toward higher drug concentrations in vaginal secretions but not in plasma. While increases in vaginal drug levels due to DMPA require confirmation, it is tempting to surmise that prolonged vaginal thinning and increased epithelial permeability induced by MPA may have resulted in higher vaginal drug levels and thus, contributed to the high protection despite possible infiltration of activated target cells. This mechanism was also proposed as an potential explanation for the high and sustained vaginal absorption of FTC and TFV from vaginal gels seen in macaques treated with 30 mg of DMPA [16].

We also note potential limitations in our study. First, virus challenges were non-traumatic and done in the absence of other cofactors that may increase HIV transmission risk such as sexually transmitted infections. Second, the shorter half-life of MPA in macaques compared to humans implied the need for monthly injections to efficiently model plasma MPA levels seen in women receiving 150 mg every 3 months. It is not known if shorter DMPA cycles resulting in more frequent MPA peaks and valleys might have affected our results.

A recent sub-analysis of participants from the Partners PrEP Study that evaluated the efficacy of TDF or FTC/TDF for HIV-1 prevention found no evidence for a reduced efficacy of PrEP among women who used DMPA and men whose female partners used DMPA [17]. Although these findings are not from a randomized clinical trial of DMPA and PrEP, they help support our observations in the macaque model, and also suggest that PrEP use in high HIV risk areas might overcome potential detrimental effects of DMPA on HIV-1 susceptibility [17]. Animal model studies such as the one described here are well suited to evaluate multipurpose prevention technologies that incorporate PrEP and contraceptives.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. United Nations. United Nations; 2011. World Contraceptive Use 2011. In. http://www.un.org/esa/population/publications/contraceptive2011/wallchart_front.pdf
2. Polis CB, Phillips SJ, Curtis KM. Hormonal contraceptive use and female-to-male HIV transmission: a systematic review of the epidemiologic evidence. AIDS. 2013; 27:493–505. [PubMed: 23079808]
3. Polis CB, Curtis KM. Use of hormonal contraceptives and HIV acquisition in women: a systematic review of the epidemiological evidence. Lancet Infect Dis. 2013; 13:797–808. [PubMed: 23871397]
4. Chandra N, Thurman AR, Anderson S, Cunningham TD, Yousefieh N, Mauck C, Doncel GF. Depot medroxyprogesterone acetate increases immune cell numbers and activation markers in human vaginal mucosal tissues. AIDS Res Human Retrov. 2013; 29:592–601.
5. Mauck CK, Callahan MM, Baker J, Arbogast K, Veazey R, Stock R, Pan Z, Morrison CS, Chen-Mok M, Archer DF, Gabelnick HL. The effect of one injection of Depo-Provera on the human vaginal epithelium and cervical ectopy. Contraception. 1999; 60:15–24. [PubMed: 10549448]
6. Mitchell CM, McLemore L, Westerberg K, Astronomo R, Smythe K, Gardella C, Mack M, Magaret A, Patton D, Agnew K, McElrath MJ, Hladik F, Eschenbach D. Long-term Effect of Depot Medroxyprogesterone Acetate on Vaginal Microbiota, Epithelial Thickness and HIV Target Cells. J Infect Dis. 2014; 210:651–655. [PubMed: 24652495]
7. Bahamondes MV, Castro S, Marchi NM, Marcovici M, Andrade LA, Fernandes A, Bahamondes L. Human vaginal histology in long-term users of the injectable contraceptive depot-medroxyprogesterone acetate. Contraception. 2014; 90:117–122. [PubMed: 24613369]
8. Radzio J, Hanley K, Mitchell J, Ellis S, Deyounks F, Jenkins LT, Hanson D, Heneine W, Garcia-Lerma JG. Physiologic doses of depot-medroxyprogesterone acetate do not increase acute plasma SHIV viremia or mucosal virus shedding in pigtail macaques. AIDS. 2014 in press.
9. Baeten J, Celum C. Oral antiretroviral chemoprophylaxis: current status. Curr Opin HIV AIDS. 2012; 7:514–519. [PubMed: 22964886]
10. Radzio J, Aung W, Holder A, Martin A, Sweeney E, Mitchell J, Bachman S, Pau C, Heneine W, Garcia-Lerma JG. Prevention of vaginal SHIV transmission in macaques by a coitally-dependent Truvada regimen. PLoS One. 2012; 7:e50632. [PubMed: 23226529]
11. Nanda K, Amaral E, Hays M, Viscola MA, Mehta N, Bahamondes L. Pharmacokinetic interactions between depot medroxyprogesterone acetate and combination antiretroviral therapy. Fertil Steril. 2008; 90:965–971. [PubMed: 17880953]
12. Steiner RA, Schiller HS, Illner P, Blandau R, Gale CC. Sex hormones correlated with sex skin swelling and rectal temperature during the menstrual cycle of the pigtail macaque (Macaca nemestrina). Lab Anim Sci. 1977; 27:217–221. [PubMed: 404463]
13. García-Lerma JG, Otten RA, Qari SH, Jackson E, Cong M, Masciotra S, et al. Prevention of rectal SHIV transmission in macaques by daily or intermittent prophylaxis with emtricitabine and tenofovir. PLoS Med. 2008; 5:e28. [PubMed: 18254653]

14. Gao WY, Shirasaka T, Johns DG, Broder S, Mitsuya H. Differential phosphorylation of azidothymidine, dideoxycytidine, and dideoxyinosine in resting and activated peripheral blood mononuclear cells. J Clin Invest. 1993; 91:2326–2333. [PubMed: 8387546]

15. García-Lerma JG, Aung W, Cong ME, Zheng Q, Youngpairoj AS, Mitchell J, Holder A, Martin A, Kuklenyik S, Luo W, Lin CY, Hanson DL, Kersh E, Pau CP, Ray AS, Rooney JF, Lee WA, Heneine W. Natural substrate concentrations can modulate the prophylactic erfficacy of nucleotide HIV reverse transcriptase inhibitors. J Virol. 2011; 85:6610–6617. [PubMed: 21525346]

16. Dobard, C.; Sharma, S.; West, R.; Martin, A.; Makarova, N.; Taylor, A.; Mitchell, J.; Pau, CP.; Heneine, W. Imapct of depo-provera on vaginal absorption of ARV drugs from gels in pigtail macaqaues. 20th Conference on Retroviruses and Opportunistic Infections; 2013; Abstract 985

17. Heffron, R.; Mugo, N.; Were, E.; Kiarie, J.; Bukusi, E.; Mujugira, A.; Donnell, D.; Ronald, A.; Celum, C.; Baeten, J.; for thePartners PrEP Study Team. Conference on Retroviruses and Opportunistic Infections. Boston: 2014. PrEP Is Efficacious for HIV Prevention Among Women Using DMPA for Contraception.

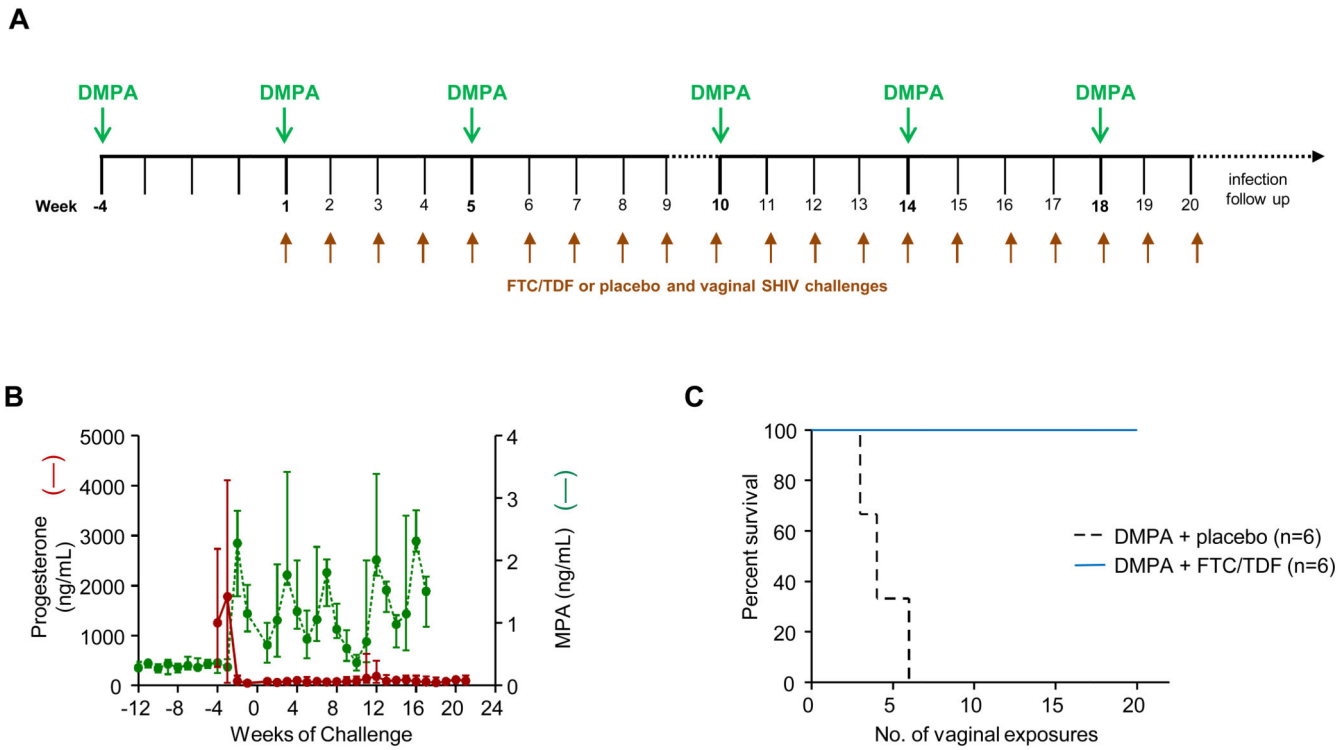

Figure 1.

Analysis of the effect of DMPA on the efficacy of PrEP with FTC/TDF. A) Experimental design. Female pigtail macaques (n = 12) received 3 mg of DMPA starting at week -4, and then at weeks 1, 5, 10, 14, and 18. Vaginal virus challenges were initiated at week 1 and continued for up to 20 weeks. During the virus challenge series, 6 animals received two weekly FTC/TDF doses 24h prior to and 2h after each virus exposure, and 6 controls received PBS. B) MPA and progesterone concentrations (median ± interquartile range) in plasma prior to and during administration of DMPA. The green line represents MPA concentrations and the red line represents progesterone levels. Progesterone levels prior to week -4 cannot be accurately represented because menstrual cycles were not yet synchronized. C) Kaplan-Meier plot. Each survival curve represents the cumulative percentage of uninfected macaques as a function of the number of vaginal exposures. We assumed an eclipse phase of 7 days to estimate time of infection from the first detection of RNA in plasma.

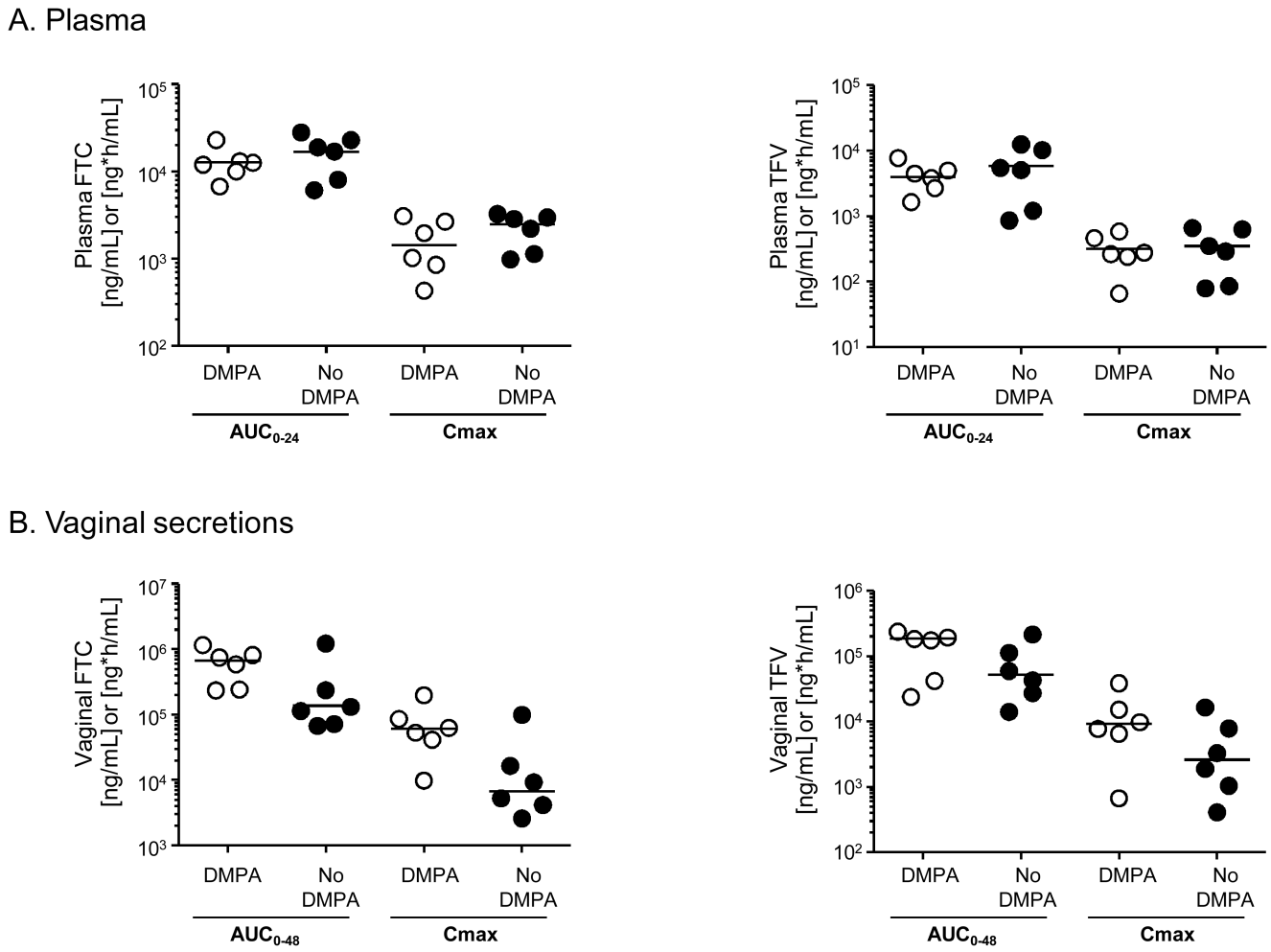

Figure 2.

Effects of DMPA on mucosal and systemic concentrations of TFV and FTC. A) C_{max} and $AUC_{0\text{-}24h}$ values for FTC and TFV obtained in plasma during DMPA treatment or during the regular menstrual cycle (no DMPA). B) C_{max} and $AUC_{0\text{-}48h}$ values for FTC and TFV obtained in vaginal secretions during DMPA treatment or during the regular menstrual cycle (no DMPA). The PK profile of FTC and TDF in DMPA treated animals was determined 2 weeks after DMPA dosing, when the concentrations of MPA in plasma are highest. Horizontal lines denote median concentrations of FTC or TFV obtained in 6 macaques.