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Sexually transmitted infections and depot medroxyprogesterone acetate do not impact protection from simian HIV acquisition by long-acting cabotegravir in macaques

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Objective: We had previously shown that long-acting cabotegravir (CAB-LA) injections fully protected macaques from vaginal simian HIV (SHIV) infection. Here, we reassessed CAB-LA efficacy in the presence of depot medroxyprogesterone acetate and multiple sexually transmitted infections (STIs) that are known to increase HIV susceptibility in women.

Design: Two macaque models of increasing vaginal STI severity were used for efficacy assessment.

Methods: The first study ($n = 11$) used a double STI model that had repeated exposures to two vaginal STI, *Chlamydia trachomatis* and *Trichomonas vaginalis*. Six animals were CAB-LA treated and five were controls. The second study ($n = 9$) included a triple STI model with repeated exposures to *C. trachomatis*, *T. vaginalis* and syphilis, and the contraceptive, depot medroxyprogesterone acetate (DMPA). Six animals were CAB-LA treated and three were controls. All animals received up to 14 vaginal SHIV challenges. A survival analysis was performed to compare the number of SHIV challenges to infection in the drug-treated group compared with untreated controls over time.

Results: All six CAB-LA treated animals in both models, the double STI or the triple STI-DMPA model, remained protected after 14 SHIV vaginal challenges, while the untreated animals became SHIV-infected after a median of two challenges (log-rank $P < 0.001$) or one challenge (log-rank $P = 0.002$), respectively. Both models recapitulated human STI disease, with vaginal discharge, ulcers, and seroconversion.

Conclusion: In these high and sustained susceptibility models spanning more than 3 months, CAB-LA maintained complete efficacy, demonstrating robustness of the CAB-LA dose used in clinical trials, and suggesting its insensitivity to multiple STIs and DMPA.

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Background

Injectable long-acting cabotegravir (CAB-LA) was highly effective in preventing HIV infection among ciswomen [HIV Prevention Trials Network (HPTN) 084] [1,2]. This trial compared the safety and efficacy of CAB-LA to daily oral tenofovir/emtricitabine (TDF/FTC) for pre-exposure prophylaxis (PrEP) and was found to be superior to TDF/FTC. These results complement data from HPTN083 [3], which showed that CAB-LA lowered HIV incidence among transgender women and cisgender men. The promise of CAB-LA is in longer dosage intervals that can improve adherence to PrEP. Nonhuman primate (NHP) modeling by our group and others preceded these clinical trial results and showed that clinically relevant doses of CAB-LA fully protect against repeated rectal, vaginal, and penile [4–6] challenges with simian HIV (SHIV). The ability of these NHP PrEP models to accurately predict clinical trials results heightens their importance in the clinical development of PrEP modalities.

According to the WHO, there are globally an estimated 131 million new cases annually of *Chlamydia*, 143 million of trichomoniasis, and six million of syphilis [7]. In the United States, the number of *Chlamydia* and syphilis cases has been on the rise, with the most recent CDC report showing additional increases from 2017 to 2018, including in women [8]. The three STIs we chose for this study were *Chlamydia trachomatis*, *Trichomonas vaginalis* and *Treponema pallidum* because of their wide prevalence, increasing incidence, and our ability to model these common STIs in NHP.

Various reports have shown that nonulcerative STIs such as *C. trachomatis* and *T. vaginalis* can result in a two to fivefold increase in HIV acquisition risk in women [9], as can ulcerative STI such as *T. pallidum* [7]. The mechanisms by which these STI increase HIV risk vary, but all likely cause elevated cytokine concentrations, an influx of activated immune cells to the vaginal milieu providing more target cells for HIV, and a compromised mucosal barrier [10]. STIs alter immune responses and, in the case of *T. pallidum*, also cause mucosal ulceration thus enabling easier HIV access to internal vasculature and immune cells [9,11]. The impact of STI on PrEP efficacy can be evaluated in animal models that recapitulate human STIs. This is difficult to evaluate in clinical trials as participants are carefully followed and treated for STIs.

We have developed vaginal NHP models of STI, including *C. trachomatis*, *T. vaginalis*, and *T. pallidum* [12,13]. The double STI model with *C. trachomatis* and *T. vaginalis* increased vaginal susceptibility to SHIV [13], likely because of prolonged inflammation in the female genital tract (FGT). In this study, we evaluated the efficacy of CAB-LA against vaginal SHIV acquisition in two macaque models of increasing STI and inflammation severity. The first is the double STI model, and the second, a triple STI-DMPA model: *C. trachomatis*, *T. vaginalis*, and syphilis (*T. pallidum*) coinfection, with additional administration of the progestin-based contraceptive, medroxyprogesterone acetate (DMPA). In NHP models, DMPA increases risk of SIV [14] and SHIV [15] acquisition by thinning macaque vaginal epithelium, reducing its barrier function [15], increasing target cells, and altering mucosal pH and mucus properties [15,16]. Both models in this study recapitulate human STI, with discharge, ulcers, and *T. pallidum* seroconversion [12,13,17].

Materials and methods

Sexually transmitted infection macaque models

All animal procedures were approved by the Centers for Disease Control and Prevention (CDC) Institutional Animal Care and Use Committee. Fourteen pigtailed macaques between the ages of 5 and 14 years (median, 12 years) were housed at CDC under the care of veterinarians in accordance with the Guide for the Care and Use of Laboratory animals.

Double sexually transmitted infection model

All 11 animals were coinfected with two STIs, *C. trachomatis* and *T. vaginalis* (Fig. 1a). The macaques were exposed to *C. trachomatis* serovar E (strain UW-447; 1×10^6 inclusion-forming units) at week −4, followed by exposure to a combination of *C. trachomatis* and *T. vaginalis* (strain Balt-42; 5×10^6 trichomonads) at week −3 and −1, before vaginal $SHIV_{SF162P3}$ challenges at week 0. We used six animals in the CAB-LA (50 mg/kg) treatment arm (animal #1–6) and five in the control arm (animal #7–11). The median animal weight was 8 kg in both the CAB-LA and control arms. *T. vaginalis* was propagated in culture and applied directly to the vaginal mucosa as previously described [18]. The animals received *C. trachomatis* and *T. vaginalis* boosts every 3 weeks.

Triple sexually transmitted infection - depot medroxyprogesterone acetate model

Six SHIV uninfected animals from the double STI model were rolled over into the treatment arm after complete drug washout, and three additional untreated controls were added (animal #12–14) (Fig. 1b). The median animal weight was 9 and 7 kg, respectively, in the two arms. All six animals in the treatment arm were exposed to *C. trachomatis*, *T. vaginalis*, and 10^8 organisms/ml *T. pallidum* Nichols strain (200 μl; four submucosal injections) at week −5 and −2, and received 1.5 mg/kg of DMPA at week −5 and −1 before vaginal SHIV challenges at week 0. The three animals in the control arm were exposed to *C. trachomatis*, *T. vaginalis*, and *T. pallidum* at week −2 and given DMPA at week −1.

Macaques were treated with DMPA every 4 weeks thereafter, and received *C. trachomatis* boosts every 3 weeks, and *T. vaginalis* every week to maintain infection. The *T. vaginalis* exposures were more frequent because DMPA treatment might affect vaginal *T. vaginalis* take in macaques (data not shown).

Sexually transmitted infection diagnosis and immunohistochemistry

The STI infection status for *C. trachomatis*, *T. vaginalis* was monitored with the APTIMA Combo 2 assay (Hologic, San Diego, California, USA). Serum antibodies showing syphilis infections were determined using *T. pallidum* particle agglutination (TP-PA) and Rapid Plasma Reagin (RPR) assays as described before [17]. Pinch biopsies from suspected vaginal syphilitic lesions collected via colposcopy were fixed in 10% neutral buffered formalin. These biopsies were routinely processed, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E). Inflammation within the biopsies was visually assessed by a veterinary pathologist. An immunohistochemical (IHC) assay targeting Spirochaetaceae (Abcam, Cambridge, Massachusetts, USA), including *T. pallidum*, was performed using protein kinase antigen retrieval and indirect immunoalkaline phosphatase detection on 4 μm sections, with colorimetric detection using the Mach 4 AP Polymer kit (Biocare Medical, Concord, California, USA) and Fast Red Chromogen (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at room temperature. Appropriate negative control serum was run in parallel. Slides were counterstained with Mayer's hematoxylin (Poly Scientific, Bay Shore, New York, USA) and blued in lithium carbonate (Polysciences, Inc., Warrington, Pennsylvania, USA). Positive controls included formalin-fixed, paraffin-embedded spirochete cultures.

Simian HIV challenge and diagnosis

Animals were challenged two times a week with SHIV ($50TCID_{50}$) using the previously described repeat low-dose model [5]. The cell-free challenge virus stock used in this study was derived from CCR5-utilizing $SHIV_{SF162P3}$ obtained originally from the NIH AIDS Reagent Repository. Briefly, $SHIV_{SF162P3}$ was amplified in pigtail macaque (*Macaca nemestrina*) peripheral blood mononuclear cells (PBMCs) following in-vitro depletion of $CD8^+$ cells (Dynabeads, CD8; Thermo Fisher Scientific) and stimulation with Concanavalin-A (Sigma-Aldrich), then clarified via centrifugation. An in-house real-time PCR assay was used to measure SHIV RNA levels in plasma as previously described [19]. Two successive SHIV RNA–positive plasma samples were required to consider an animal SHIV-infected and ceasing viral challenges. We used a 7-day eclipse period to define the SHIV challenge number that caused infection in the controls.

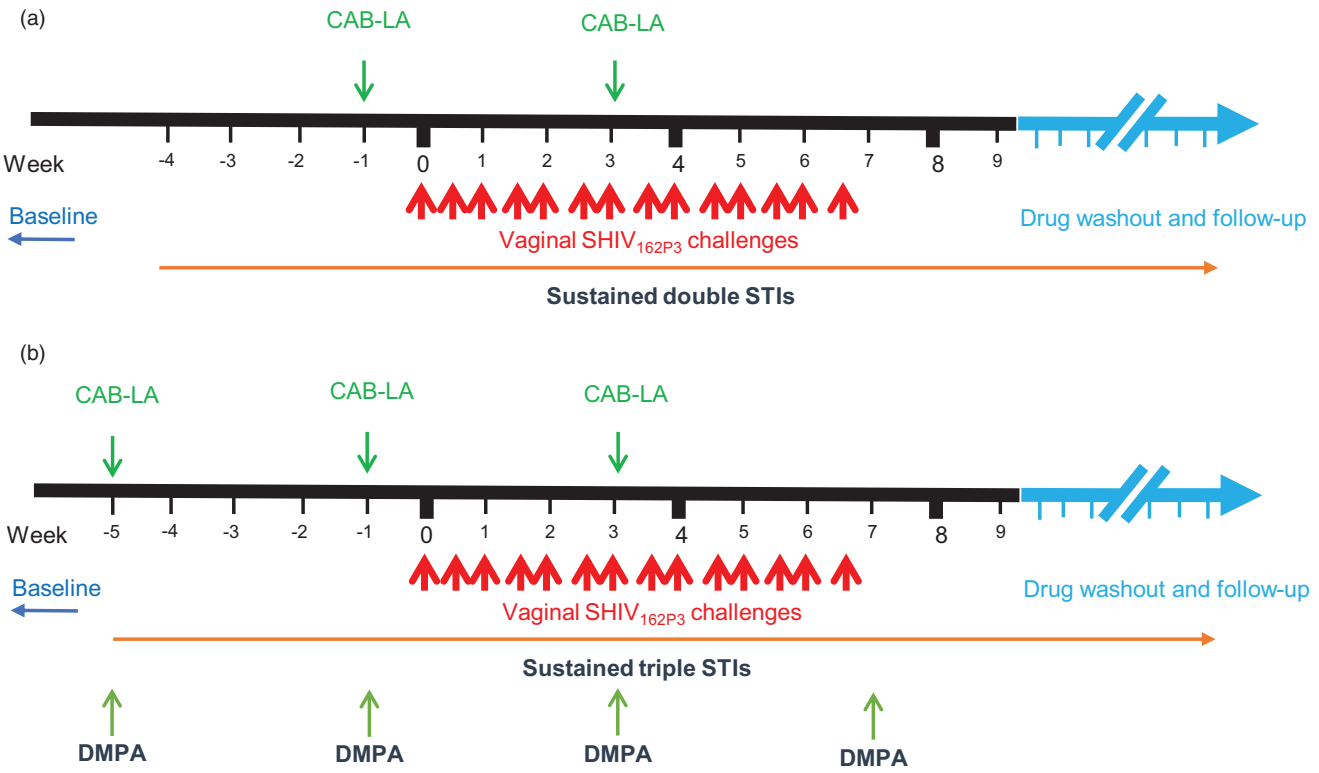

Fig. 1. Study design in double sexually transmitted infection (a) and triple STI-DMPA (b) models. In both models, all animals were vaginally challenged with SHIV twice a week beginning at week 0. The treatment groups were administered 50 mg/kg CAB-LA every 4 weeks. STI, sexually transmitted infection.

Long-acting cabotegravir, drug pharmacokinetic analyses, and progesterone

CAB-LA (200 mg/ml injectable stock aqueous suspension) was provided by ViiV Healthcare (Research Triangle Park, North Carolina, USA) and administered at a 50 mg/kg dose every 4 weeks (Fig. 1) under anesthesia by intramuscular injection into the quadriceps muscle. This dosing in macaques maintains plasma drug concentrations similar to those in humans given 600 mg intramuscular injections every 8 weeks [4]. Volumes greater than 1.0 ml were divided between two injection sites. CAB-LA concentrations were determined using liquid chromatography coupled with tandem mass spectrometry in blood plasma, and rectal and vaginal secretions that were frozen at −80°C before analysis. We did not measure drug levels in the vaginal secretions from macaques during the SHIV challenge phase to avoid potential mucosal disruption. The assay had a lower quantification limit of 7 ng/ml for swabs and 10 ng/ml for plasma. On the basis of the maximum retention of PBS we measured in polyester swabs (Thermo Fisher Scientific), we estimated a swab contains 140 μl and used this volume to convert ng/swab measurements to ng ml^{-1}. Plasma progesterone levels were monitored during baseline and throughout the study to determine the menstrual status of the animals. Progesterone evaluation was done at the Wisconsin National Primate Research Center.

Statistical analyses

A Kaplan–Meier graph was used to visually inspect number of SHIV challenges to infection in the double STI model treatment arm relative to the control arm, and the triple STI-DMPA model treatment arm relative to the control arm. The log-rank test was used to compare survival distributions. Using the Wilcoxon rank sum test, the differences in median number of challenges were evaluated between untreated (control) animals in the two models, and historical (non-STI; $n = 16$) controls that had received the same repeat low-dose challenges with the same stock (data not published). Pharmacokinetic data were $\log_{10}$ transformed and an area under the curve (AUC) was computed for each monkey using nested arrays and the trapezoidal rule [20]. We then compared AUCs between the two models for plasma, rectal and vaginal specimens using the Wilcoxon rank sum test. For the plasma pharmacokinetic comparisons, we included all time points after the final drug injections. SAS version 9.4 (SAS Institute, Inc., Cary, North Carolina, USA) was used for all analyses.

Results

Preexposure prophylaxis efficacy of long-acting cabotegravir

In the double STI model, the six drug-treated STI-infected animals remained protected after 14 SHIV exposures, while all five untreated animals became SHIV-infected (time to event analysis, log-rank $P < 0.001$; Fig. 2a) after a median of two challenges (range 1–10; see Figure, Supplemental Digital Content 1, http://links.lww.com/QAD/C269). Likewise, in the triple STI-DMPA model, despite the addition of *T. pallidum* and DMPA, the six drug-treated STI-infected animals remained protected after 14 SHIV challenges, while all three untreated animals became SHIV-infected ($P = 0.002$; Fig. 2b) after a median of one challenge (range 1–3; see Figure Supplemental Digital Content 1, http://links.lww.com/QAD/C269).

Drug pharmacokinetic evaluation

We measured CAB levels in plasma in all treated animals to ascertain if drug concentrations in our study were above 4x protein adjusted 90% inhibitory concentration (PA-IC90; 664 ng/ml), a target level in clinical PrEP trials and previously conducted macaque studies that showed high efficacy against vaginal SHIV challenges [7]. We also wanted to compare the relative drug levels in plasma, vaginal, and rectal secretions in animals from both models. The levels in the vaginal secretions provide an understanding of mucosal drug exposures during STIs. The levels in the rectum were measured to determine intercompartment differences in drug concentrations. The mean drug concentrations in plasma were above 664 ng/ml at each SHIV challenge in both models (Fig. 3), remaining above this level in the double STI model and triple STI-DMPA model for up to 6 and 5 weeks, respectively, following the last CAB-LA injection. The median CAB levels in vaginal secretions from both models were below 1x PA-IC90 (166 ng/ml), except for two of 14 time points in the double STI model (weeks 8 and 9; Fig. 3) and 1 of 17 time points in the second model (week 3). In both models, the median CAB levels in the rectal secretions were above 1x PA-IC90 for up to 7 weeks after the last drug injection. In terms of relative concentrations in the two models, the median drug levels in plasma over 8 weeks after the last CAB-LA injection were three to fivefold higher than those in rectal

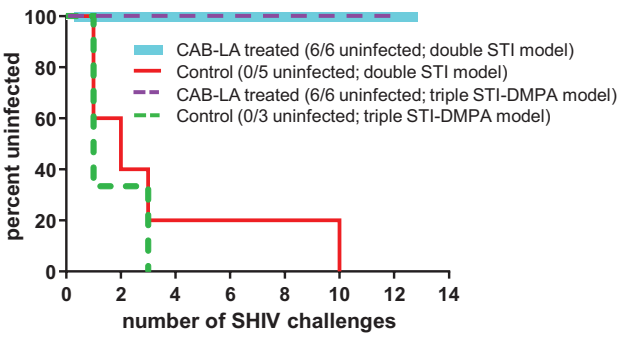

Fig. 2. Kaplan–Meier plots in double STI (log-rank $P < 0.001$) and triple STI-DMPA ($P = 0.002$) models. Each survival curve represents the cumulative percentage of uninfected animals as a function of the number of SHIV Challenges. STI, sexually transmitted infections.

secretions, and these drug levels were 13 to 15-fold higher than levels in vaginal secretions (Fig. 3).

To make sure administration of DMPA suppressed ovulation, we measured plasma progesterone levels in the animals in addition to levels of MPA. The systemic MPA levels peaked at week 2 after DMPA administration and endogenous progesterone levels dropped to undetectable in most animals by week 2, and in all animals by week 4 after the first DMPA administration (see Figure, Supplemental Digital Content 2, http://links.lww.com/QAD/C270), indicating full suppression of ovulation. Because a prior study using DMPA in rhesus macaques [21] had reported that DMPA might alter CAB levels, we compared CAB levels in the DMPA and non-DMPA treated animals. The calculated *P* value (0.10) suggested that DMPA administration (or *T. pallidum*) did not affect the pharmacokinetic profile of plasma CAB (see Figure, Supplemental Digital Content 3, http://links.lww.com/QAD/C271) in the triple STI-DMPA model.

Sexually transmitted infection detection, serology, and immunohistochemistry of vaginal lesions

To confirm *C. trachomatis*, *T. vaginalis* infections, we looked at cervicovaginal manifestations prior to starting CAB-LA administration and SHIV challenges. Representative figures are shown in supplemental data (see Figure, Supplemental Digital Content 4, http://links.lww.com/QAD/C272). During the study, we confirmed *C. trachomatis*, *T. vaginalis* infection status by evaluating the presence of pathogen in vaginal secretions. All 11 animals in the double STI model showed vaginal CT infections before commencement of SHIV challenges (see Table 1a, Supplemental Digital Content, http://links.lww.com/QAD/C273), while in the triple STI-DMPA model, all nine animals except animal #14, showed *C. trachomatis* infections (see Table 1b, Supplemental Digital Content, http://links.lww.com/QAD/C273). Animal #14 showed positive *C. trachomatis* status in subsequent assays performed after completion of SHIV exposures. Before beginning SHIV challenges, *T. vaginalis* infections in the double STI were established in five out of six treated animals and three out of five control animals; the animals that tested negative for *T. vaginalis* before SHIV challenges exhibited successful take of *T. vaginalis* infection when tested later after the SHIV challenge phase (see Table 1A, Supplemental Digital Content, http://links.lww.com/QAD/C273). In the triple STI-DMPA model, *T. vaginalis* infections were established in one out of six treated animals and one out of three control animals (see Table 1b, Supplemental Digital Content, http://links.lww.com/QAD/C273). To document syphilitic lesions, frequently ulcerative, we used colposcopy and narrow band imaging [12] in four drug-treated animals on day 7 and day 14 following first *T. pallidum* exposure. Representative images are shown in Fig. 4a, b. Our previous data (not shown) indicate that vaginal syphilitic lesions appear 7–14 days (mean, 12 days) after *T. pallidum* exposure. The lesions persist for up to 87 days (mean, 56 days), which overlap the SHIV-challenge period in this study. To document lesions and inflammation in the triple STI-DMPA model, we performed colposcopy and vaginal biopsies were collected from three control animals at 7 weeks following *T. pallidum* exposure. Colposcopy revealed lesions and the H&E staining showed dense lymphoplasmacytic inflammation and lymphoid follicles (See examples in Fig. 4c, d). Syphilis infections were confirmed by using TP-PA and RPR assays to detect seroconversion in all animals. Representative antibody titers from one animal are shown in Table 2, Supplemental Digital Content, http://links.lww.com/QAD/C274.

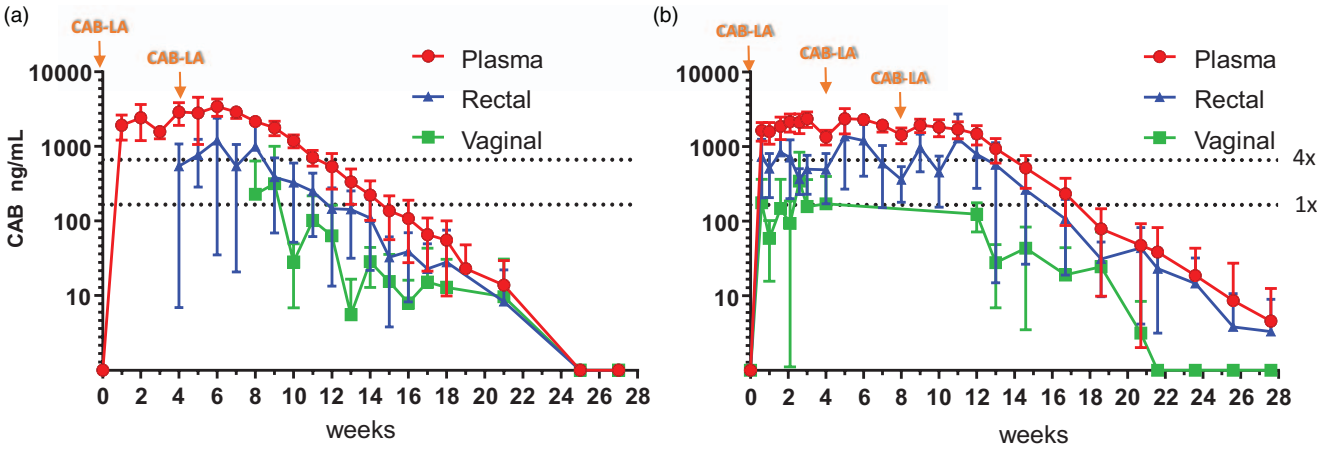

Fig. 3. CAB concentrations in blood plasma, and rectal and vaginal secretions in the double STI (a) and triple STI-DMPA (b) models. Values denote means ± SEM. Horizontal dotted lines denote 1x and 4x PA-IC_{90}. Week 0 = first CAB-LA injection. In the triple STI-DMPA model, we could collect vaginal samples from week 1–4 following first CAB-LA injection, as the SHIV challenges started only after the second CAB-LA injection. In the double STI model, the SHIV challenges started 1 week after first CAB-LA injection. STI, sexually transmitted infection.

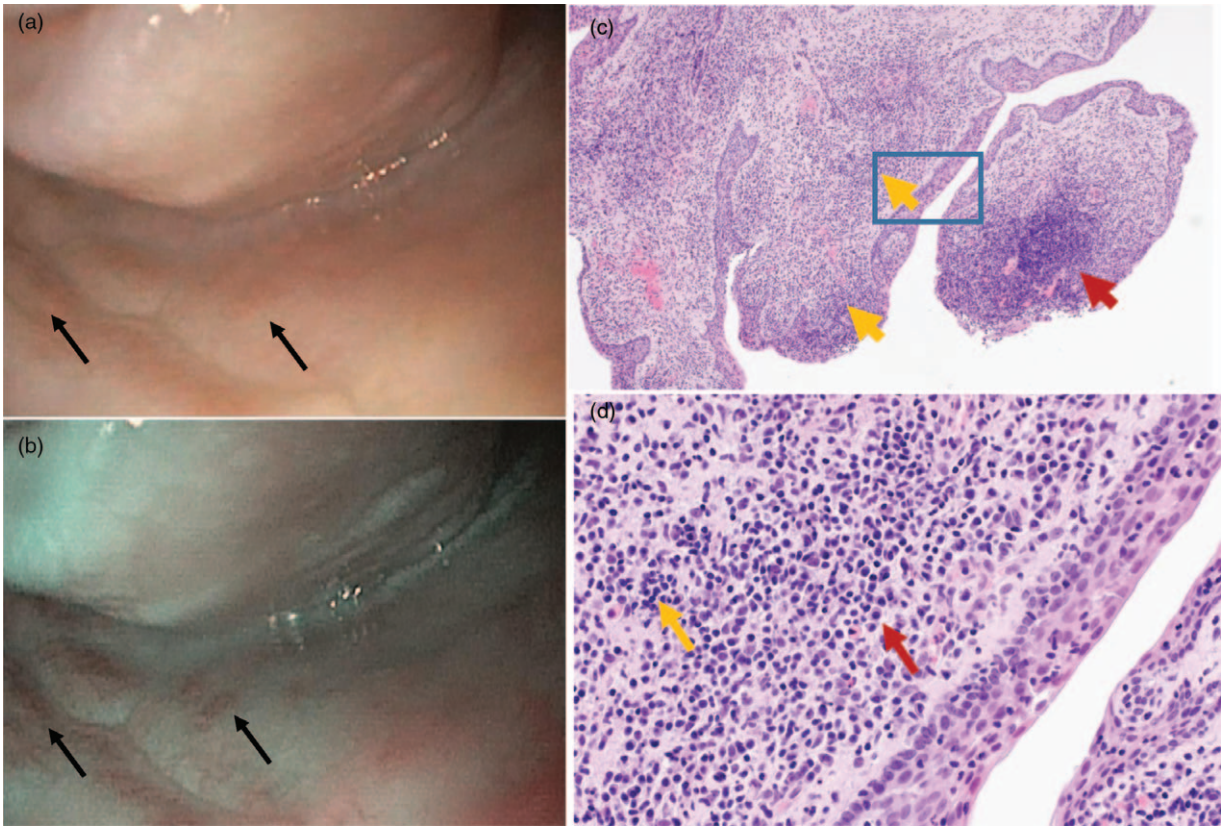

Fig. 4. Colposcopy and hematoxylin and eosin staining. Colposcopy was done on four drug-treated animals on day 7 and day 14 following first TP exposure to look for syphilitic lesions. Shown are representative colposcopy pictures of lesions in the vaginal wall of pig-tailed macaque (animal #4) taken at day14 following submucosal *T. pallidum* exposure using regular (a) and narrow band (b) imaging; H&E stain of vaginal biopsies at 5x magnification (c) from one animal (animal #12; untreated control) collected at week 7 demonstrating areas of dense lymphoplasmacytic inflammation (yellow arrows) and lymphoid follicles (red arrow); (d) Enlarged view of the same field (rectangular box) at 20x magnification, populated predominately with plasma cells (red arrow) and lymphocytes (yellow arrow); there is no disruption of architecture. We did not evaluate vaginal thinning in the collected tissues.

Discussion

On the basis of its high clinical efficacy and safety, it is anticipated that CAB-LA will be approved soon for HIV prevention in men and women. In agreement with the clinical findings, we have previously demonstrated that a clinical CAB-LA fully protected against vaginal SHIV infection in macaques [5]. Using a similar SHIV challenge strategy and CAB-LA dose, we have assessed in this study CAB-LA efficacy in two STI models of increased severity. We show that CAB-LA maintained full protection reflecting high robustness of the CAB-LA dose and its insensitivity to STI/DMPA mediated inflammation, ulceration or vaginal thinning. The findings are reassuring and support the current clinical CAB-LA dose. The study has benefited from the use of STI models that recapitulate clinical infections, combine ulcerative and nonulcerative STIs, and are augmented by DMPA that facilitates SHIV acquisition in macaques. The models were designed to sustain STI manifestations over 3 months by repeat STI exposures which overlapped repetitive physiologic SHIV challenges.

The findings observed with CAB-LA do not parallel those we observed when TDF/FTC efficacy was tested in the double *C. trachomatis* / *T. vaginalis* STI model [22]. Although TDF/FTC maintained significant protection, two out of six macaques became SHIV-infected compared with zero out of six in the absence of STI. It is unclear why CAB-LA efficacy was not similarly impacted. It is possible that the CAB-LA dose used is very potent and able to overcome any STI-mediated increase in SHIV infection and spread. In fact, previous work in the macaque model defined the correlate of protection by showing plasma CAB concentrations more than 3x PA-IC90 conferred 100% protection, whereas concentrations at least 1x PA-IC90 conferred nearly 97% protection [4]. Thus, the demonstration of plasma CAB concentrations persisting above 4x PA-IC90 in all animals supports the idea of a highly potent PrEP dose. Also, the detection of CAB in the vaginal secretions from the STI-infected animals, although lower than plasma levels, is also important because it suggests mucosal antiviral activity that may have contributed to protection. It is also possible that the drug class and its mechanism of action maybe

differently affected by the mucosal environment. Unlike CAB, which is an integrase inhibitor, both tenofovir and FTC are nucleotide analogs of dATP and dCTP, respectively, and maybe sensitive to competition by higher intracellular natural substrates that are commonly increased in activated target cells during inflammation. Indeed, our prior studies in the double STI model showed elevated dATP and dCTP concentrations in vaginal tissues [22], suggesting a possible reduction in local antiviral activity. One limitation of this study is the lack of consistent *T. vaginalis* infections, especially in the presence of DMPA, which may have resulted in lower than expected levels of vaginal inflammation. The impact of inconsistent *T. vaginalis* infection may, however, be limited in the presence of consistent infection with *C. trachomatis*, *T. pallidum*, and a DMPA effect. We have not evaluated the combination of syphilitic manifestation or DMPA singly with CT-TV coinfection, and therefore cannot determine the individual contributions of syphilis and DMPA.

In a prior study of DMPA-treated rhesus macaques, lower systemic concentrations of CAB were observed than in non-DMPA treated macaques [21]. One mechanism proposed was increased metabolism of CAB due to upregulation of components of the hepatic glucuronidation pathway by progesterone. However, these studies also compared male and female macaques, raising the possibility of sex differences in CAB metabolism, and used a high DMPA dose (30 mg per macaque or approximately 3 mg/kg). In our experiment, we were able to directly compare CAB concentrations in the same female macaques before and after DMPA administration. We used lower doses of DMPA (1.5 mg/kg) which our prior work [15] suggests is a more physiologic dose that suppresses ovulation and causes significant thinning of vaginal epithelium. We show no impact by DMPA on plasma CAB concentrations obviating the need for dose modification.

In summary, we demonstrate in two macaque models robustness of the CAB-LA clinical dose and its insensitivity to multiple STIs and DMPA supporting the implementation of CAB-LA for HIV prevention in women. Our STI-DMPA models provide an additional preclinical tool to confirm efficacy and dose selection of novel PrEP modalities.

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Conceived and designed the experiments: S.A.V., J.M.M., W.H., J.G.G.-L., W.R.S. Performed the experiments: C.Z., R.L., M.M.M., C.D., M.J.G., J.M., L.E.P. Analyzed the data: S.A.V., C.Z., G.K.K., R.L. Wrote the article: S.A.V., J.M.M., W.H. The authors thank David Garber for programmatic support; Shanon Ellis, Ryan Johnson, Frank Deyounks, and Kristen Kelley for animal procedure assistance; our veterinarians and colleagues in the Comparative Medicine Branch (CMB), for help with macaque-related work; stocks of *Chlamydia trachomatis* (serovar E; strain UW-447) were obtained from University of Washington; $SHIV_{SF162P3}$ was obtained through the NIH AIDS Reagent Program, NIAID, NIH from Janet Harouse, Cecilia Cheng-Mayer, Ranajit Pal, and the DAIDS/NIAID. The long-acting cabotegravir was obtained from ViiV Healthcare (Research Triangle Park, North Carolina, USA).

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Conflicts of interest

W.H., J.G.G.-L. are named on patents and patent applications by the US government on methods for HIV prevention by chemoprophylaxis. W.S. is an employee of GSK/ViiV.

The findings and conclusions in this paper are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention. Use of trade names is for identification only and does not imply endorsement by the U.S. Department of Health and Human Services, the Public Health Service, or the Centers for Disease Control and Prevention.

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References

1. Moretlwe DS, Hughes J, Bock P, Gurrion S, Hunidzarira P, Kalonjiet D, *et al*. **Long acting injectable cabotegravir is safe and effective in preventing HIV infection in cisgender women: interim results from HPTN 084.** *J Int AIDS Soc* 2021; **24**:8.
2. HIV Prevention Trials Netwoerk (HPTN). November 9, 2020. HPTN 084 Study Demonstrates Superiority of CAB LA to Oral FTC/TDF for the Prevention of HIV. Available at https://www.hptn.org/news-and-events/press-releases/hptn-084-study-demonstrates-superiority-of-cab-la-to-oral-ftctdf-for [Accessed 14 June 2021]
3. HIV Prevention Trials Netwoerk (HPTN). May 18, 2020. HPTN 083 Long-acting injectable cabotegravir is highly effective for the prevention of HIV infection in cisgender men and transgender women who have sex with men. Available at https://www.hptn.org/news-and-events/press-releases/long-acting-injectable-cabotegravir-highly-effective-prevention-hiv [Accessed 14 June 2021]
4. Andrews CD, Spreen WR, Mohri H, Moss L, Ford S, Gettie A, *et al*. **Long-acting integrase inhibitor protects macaques from intrarectal simian/human immunodeficiency virus.** *Science* 2014; **343**:1151–1154.
5. Radzio J, Spreen W, Yueh YL, Mitchell J, Jenkins L, Garcia-Lerma JG, *et al*. **The long-acting integrase inhibitor GSK744 protects macaques from repeated intravaginal SHIV challenge.** *Sci Transl Med* 2015; **7**:270ra275.

6. Dobard C, Makarova N, Nishiura K, Dinh C, Holder A, Sterling M, *et al*. **Long-acting cabotegravir protects macaques against repeated penile simian-human immunodeficiency virus exposures.** *J Infect Dis* 2020; **222**:391–395.
7. Newman L, Rowley J, Vander Hoorn S, Wijesooriya NS, Unemo M, Low N, *et al*. **Global estimates of the prevalence and incidence of four curable sexually transmitted infections in 2012 based on systematic review and global reporting.** *PLoS One* 2015; **10**:e0143304.
8. Centers for Disease Control and Prevention. October 8, 2019. New CDC Report: STDs Continue to Rise in the U.S. Available at https://www.cdc.gov/nchhstp/newsroom/2019/2018-STD-surveillance-report-press-release.html [Accessed 14 June 2021]
9. Fleming DT, Wasserheit JN. **From epidemiological synergy to public health policy and practice: the contribution of other sexually transmitted diseases to sexual transmission of HIV infection.** *Sex Transm Infect* 1999; **75**:3–17.
10. Mwatelah R, McKinnon LR, Baxter C, Abdool Karim Q, Abdool Karim SS. **Mechanisms of sexually transmitted infection-induced inflammation in women: implications for HIV risk.** *J Int AIDS Soc* 2019; **22 (Suppl 6)**:e25346.
11. Wall KM, Karita E, Nyombayire J, Ingabire R, Mukamuyango J, Parker R, *et al*. **Genital abnormalities, hormonal contraception, and HIV transmission risk in Rwandan serodifferent couples.** *J Infect Dis* 2021; **224**:81–91.
12. Vishwanathan SA, Zhao C, Luthra R, Howard R, Mills P, Morris M, *et al*. **A nonhuman primate model for vaginally acquired syphilis**. *Annual Symposium on NHP Models for AIDS*; San Antonio, TX, November 12–15, 2019.
13. Henning TR, Butler K, Hanson D, Sturdevant G, Ellis S, Sweeney EM, *et al*. **Increased susceptibility to vaginal simian/human immunodeficiency virus transmission in pig-tailed macaques coinfected with Chlamydia trachomatis and Trichomonas vaginalis.** *J Infect Dis* 2014; **210**:1239–1247.
14. Sodora DL, Gettie A, Miller CJ, Marx PA. **Vaginal transmission of SIV: assessing infectivity and hormonal influences in macaques inoculated with cell-free and cell-associated viral stocks.** *AIDS Res Hum Retroviruses* 1998; **14 (Suppl 1)**:S119–123.
15. Butler K, Ritter JM, Ellis S, Morris MR, Hanson DL, McNicholl JM, *et al*. **A depot medroxyprogesterone acetate dose that models human use and its effect on vaginal SHIV acquisition risk.** *J Acquir Immune Defic Syndr* 2016; **72**:363–371.
16. Carias AM, Allen SA, Fought AJ, Kotnik Halavaty K, Anderson MR, Jimenez ML, *et al*. **Increases in endogenous or exogenous progestins promote virus-target cell interactions within the nonhuman primate female reproductive tract.** *PLoS Pathog* 2016; **12**:e1005885.
17. Tansey C, Zhao C, Hopkins A, Ritter JM, Fakile YF, Pillay A, *et al*. **A nonhuman primate model for rectally transmitted syphilis.** *J Infect Dis* 2018; **217**:1139–1144.
18. Henning T, Fakile Y, Phillips C, Sweeney E, Mitchell J, Patton D, *et al*. **Development of a pigtail macaque model of sexually transmitted infection/HIV coinfection using Chlamydia trachomatis, Trichomonas vaginalis, and SHIV(SF162P3).** *J Med Primatol* 2011; **40**:214–223.
19. Subbarao S, Otten RA, Ramos A, Kim C, Jackson E, Monsour M, *et al*. **Chemoprophylaxis with tenofovir disoproxil fumarate provided partial protection against infection with simian human immunodeficiency virus in macaques given multiple virus challenges.** *J Infect Dis* 2006; **194**:904–911.
20. Pannucci M. Computing Area Under The Curve (AUC) Using Nested Arrays. NorthEast SAS Users Group (NESUG) 11–14 September 2011. Available at https://www.lexjansen.com/nesug/nesug11/ds/ds07.pdf.
21. Andrews CD, Yueh YL, Spreen WR, St Bernard L, Boente-Carrera M, Rodriguez K, *et al*. **A long-acting integrase inhibitor protects female macaques from repeated high-dose intravaginal SHIV challenge.** *Sci Transl Med* 2015; **7**:270ra274.
22. Radzio J, Henning T, Jenkins L, Ellis S, Farshy C, Phillips C, *et al*. **Combination emtricitabine and tenofovir disoproxil fumarate prevents vaginal simian/human immunodeficiency virus infection in macaques harboring Chlamydia trachomatis and Trichomonas vaginalis.** *J Infect Dis* 2016; **213**:1541–1545.