Depot Medroxyprogesterone Acetate (DMPA) Use is Associated with Elevated Innate Immune Effector Molecules in Cervicovaginal Secretions of HIV-1-uninfected Women

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Abstract

OBJECTIVE—The effects of sex hormones on the immune defenses of the female genital mucosa and its susceptibility to infections are poorly understood. The injectable hormonal contraceptive depot medroxyprogesterone acetate (DMPA) may increase risk of HIV-1 acquisition. We assessed the local concentration in the female genital mucosa of cationic polypeptides with reported antiviral activity in relation to DMPA use.

METHODS—HIV-1-uninfected women were recruited from among couples testing for HIV in Nairobi, Kenya. Cervicovaginal secretion (CVS) samples were collected and the concentrations of HNP1–3, LL-37, lactoferrin, HBD-2 and SLPI were measured by enzyme-linked immunosorbent assays.. Levels of cationic polypeptides in CVS were compared between women who were not using hormonal contraception and those using DMPA, oral, or implantable contraception.

RESULTS—Among 228 women, 165 (72%) reported not using hormonal contraception at enrollment, 41 (18%) used DMPA, 16 (7%) used an oral contraceptive, and 6 (3%) used a contraceptive implant. Compared to non-users of hormonal contraception, DMPA users had significantly higher mean levels of HNP1–3 (2.38 vs. 2.04 $\log_{10}$ ng/ml; p=0.024), LL-37 (0.81 vs. 0.40 $\log_{10}$ ng/ml; p=0.027), and lactoferrin (3.03 vs. 2.60 $\log_{10}$ ng/ml; p=0.002), whereas SLPI and HBD-2 were similar.

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CONCLUSIONS—Although all analyzed cationic polypeptides have intrinsic antiviral capacity, their interaction and cumulative effect on female genital mucosa susceptibility to infections *in vivo* has yet to be unraveled. This study suggests a potential mechanism underlying the effect of DMPA on the innate immune defenses, providing a rationale to investigate its effect on HIV-1 acquisition risk.

Keywords

HIV; cationic; contraceptive; Depo-Provera; progestogen; depot medroxyprogesterone acetate

Introduction

Success of family planning initiatives, particularly in resource limited settings, depends on the wide availability of contraceptive options that are acceptable, highly effective, and safe. These requirements are partially met by long acting injectable hormonal contraceptives, most commonly in the form of depot medroxyprogesterone acetate (DMPA). In sub-Saharan Africa, DMPA accounts for the vast majority of hormonal contraceptive use.[1,2] Unfortunately, a growing body of evidence links DMPA use to increased risk of HIV-1 acquisition.[3,4] This evidence comes from observational studies, and it remains unknown what proportion of the increased risk, if any, is due to biological effects of DMPA and what proportion is attributable to residual confounding from sexual risk behavior. Identification of biological mechanisms linking DMPA use and risk of HIV infection would increase understanding of whether hormonal contraceptive use is a true biological risk factor for HIV infection.

Innate immune responses in the female genital mucosa are an important component of resistance to infectious agents. Cationic polypeptides are among the principal effector molecules of the innate immune response in the vaginal mucosa.[5] *In vitro* studies show that cationic polypeptides, including the α-defensins human neutrophil peptides 1–3 (HNP1–3), human cathelicidin antimicrobial peptide (hCAP) 18/LL-37, secretory leukocyte protease inhibitor (SLPI), lactoferrin, and human β-defensin (HBD-2), can individually inhibit a number of infectious agents, including HIV-1, through a variety of mechanisms.[6–9] These antimicrobial peptides are abundant in cervicovaginal secretions (CVS),[10,11] although their interaction *in vivo* and consequent effect on susceptibility to infections is not fully understood. Despite their intrinsic antiviral activity, higher levels of some cationic polypeptides, particularly HNP1–3 and LL-37, have been associated with increased risk of HIV infection.[12] This may be due to the recruitment by cationic polypeptide of CD4+ immune cells, which are preferentially infected by HIV-1.[13,14]

Changes in antimicrobial peptide levels in the female genital mucosa have been studied mainly in relation to the variation of sex hormones during the menstrual cycle.[15] Hormonal contraceptive agents can modulate immune factors as well,[16–19] although little work has been done to address their effect locally on the immune defenses of the female genital mucosa.[20,21] In this study, we investigated whether DMPA and other forms of hormonal contraception have an effect on the local production of five of the most abundant cationic polypeptides in the female genital mucosa (HNP1–3, LL-37, SLPI, lactoferrin, and HBD-2)

by measuring their levels in CVS from HIV-1-uninfected Kenyan women. Studying this relationship may be crucial to understanding how hormonal contraceptives could influence HIV-1 acquisition. Because HIV-1 risk is not uniform across women in the population, we also assessed whether any potential associations between hormonal contraception and these effector molecules differed between women at low-risk of HIV-1 exposure (those in HIV-1 concordant negative couples) and women who are highly exposed to HIV-1 because they are a member of an HIV-1-discordant couple. We chose to include women from HIV-1-discordant couples because they represent a group at particularly high risk of HIV-1 infection who may also be more likely to choose to use hormonal contraception. Their inclusion allowed us to determine if there were any differences between this high risk group and other low risk women in terms of potential associations between hormonal contraceptive use and levels of innate effector molecules.

Methods

Study setting and participants

This study included HIV-1-uninfected women who were recruited from voluntary counseling and testing (VCT) centers in Nairobi, Kenya from 2007 to 2009. These women were members of stable heterosexual couples who attended the VCT center with their male partner. Women included in this study were members of couples that were HIV-1-concordant negative or discordant (male partner infected and female partner uninfected). The women in this study were drawn from a parent cohort described elsewhere.[22] Briefly, eligible participants were 18 years of age, reported sexual intercourse with their study partner at least three times in the 3 months prior to screening and planned to remain together for the duration of the study (up to 24 months). We excluded couples that were enrolled in another HIV-1 treatment or prevention trial, or planned to be away from Nairobi for 2 consecutive months during follow-up. For discordant couples, we excluded those in which the HIV-1-infected partner was on antiretroviral therapy (ART) or had a history of clinical AIDS (WHO stage IV). Enrolled couples were counseled on condom use, and HIV-1-infected individuals were referred for ART evaluation if their CD4 count met national criteria for treatment initiation. All couples were screened for sexually transmitted infections (STIs) at study entry and treated. For this analysis, women were excluded if they had a hysterectomy, tubal ligation, reported currently having an intrauterine device (IUD), or were missing information on contraceptive use. Written informed consent was obtained from all study participants, and ethical approval was granted by Institutional Review Boards at University of Washington, Karolinska Institutet, and Kenyatta National Hospital.

Assessment of contraceptive use

At enrollment, women completed a sociodemographic questionnaire and provided sexual and reproductive histories. Current contraceptive use was assessed using a standardized questionnaire administered by a study nurse or clinician. Women were asked if they were currently using any form of contraception, and if so, which form(s). Women were categorized as not currently using hormonal contraception, or using DMPA, oral contraception, or implantable hormonal contraception. During the study, implantable contraception available in Kenya was the Jadelle implant, consisting of two levonorgestrel-

containing silicone rods inserted under the skin of the upper arm (Bayer Schering Pharma Oy, Berlin-Wedding, Germany).

Collection of cervicovaginal secretion samples

At study enrollment, complete physical and pelvic exams were performed. Samples of CVS were collected by rotating a cotton swab 360° in the cervical os and rotating another swab across the vaginal wall.[23] Both swabs were placed in the same tube containing 5 ml sterile phosphate buffered saline and immediately transported to the laboratory on ice. Samples were centrifuged at 800 g for 10 minutes at 4°C and supernatants were separated from the cell pellet and stored at −80°C.

Microbiological testing

HIV-1 rapid testing was conducted in clinic according to Kenyan guidelines using two commercial kits (Determine HIV1/2 Rapid Test, Abbott Laboratories; Bioline Recombigen HIV test, Standard Diagnostics Inc), with confirmation using Vironostika HIV-1 enzyme linked immunosorbant assay (ELISA) (BioMérieux Laboratories). Plasma HIV-1 RNA levels were determined using the Gen-Probe transcription mediated amplification assay, which detects the prevalent HIV-1 subtypes in Kenya, subtypes A, C, and D.[24,25] HSV-2 serostatus was determined using the HerpesSelect IgG ELISA kit (Focus Technologies), with an optical density of 1.1–3.4 defined as equivocal and >3.5 defined as positive.[26,27] Vaginal gram stains were performed and bacterial vaginosis (BV) was defined as a Nugent score of 7–10.[28]

Measurement of soluble factors in CVS samples

Concentrations of the cationic polypeptides were quantified by ELISA as previously described.[23] Briefly, commercial ELISA kits were used to quantify the immune molecules of interest at the following dilutions: HNP1–3 (1:500), LL-37 (1:10), lactoferrin (1:100–1:200) (Hycult Biotechnology, Uden, The Netherlands), HBD-2 (1:50) (Phoenix Pharmaceutical, Burlingame CA, USA), and SLPI (1:100–1:1000) (R&D Systems, Minneapolis MN, USA). The concentration of the factors in CVS are reported as measured in the original 5 ml dilution. Values that were below the lower limit of detection (LLD) were reported as the midpoint between zero and the LLD. The LLD (pg/ml) was 156.0 for HNP1–3, 100.0 for LL-37, 400 for lactoferrin, 15.6 for HBD-2, and 62.5 for SLPI.

Prostate-specific antigen (PSA) was measured from all CVS samples for this study by an external accredited laboratory (Aleris Medilab, Uppsala, Sweden) using the ARCHITECT Total PSA assay (Abbott Ireland Diagnostics Division, Sligo, Ireland), a chemoluminescent microparticle immunoassay. PSA levels in the CVS are reported as measured in the original 5 ml dilution, and positivity was defined as at least 1 ng/ml, as described previously.[29] PSA, a protein secreted during male ejaculation, can be detected in CVS with high sensitivity, and clears from CVS within 48 h with a mean clearance time of approximately 20–27 h, thus presence of PSA indicates recent unprotected sex.[29–31]

Statistical analysis

Cationic polypeptide concentrations were $\log_{10}$ transformed due to their skewed distribution. Linear regression was used to compare mean $\log_{10}$ polypeptide concentrations between categories of current hormonal contraceptive use (defined as no hormonal contraceptive use, DMPA, oral contraceptives, or implantable hormonal contraception). Other potential correlates, selected *a priori*, were also assessed. To determine if the relationship between hormonal contraceptive use at polypeptide levels differed between women from HIV-1-discordant and concordant negative couples, we included HIV-1 exposure group as an interaction term in linear regression models. A correlation matrix was calculated to assess the relationships between the cationic polypeptides. Plasma HIV-1 RNA viral levels below the lower limit of detection were set to half the lower limit (75 copies/ml). All analyses were conducted using Stata version 11 (Stata Corp., College Station).

Results

Cohort characteristics by hormonal contraceptive use

We enrolled 246 HIV-1-uninfected women, of whom 233 were eligible for this analysis (women were excluded if they reported a hysterectomy or tubal ligation [n=6], were using an intrauterine device [n=6], or were missing information on contraceptive use [n=1]) and 228 had CVS samples available to determine levels of cationic polypeptides. Of these, 158 were from discordant couples and 70 were from concordant negative couples. At enrollment 165 (72%) reported no current hormonal contraceptive use, 41 (18%) were using DMPA, 16 (7%) were using oral contraception, and 6 (3%) were using an implantable hormonal contraceptive (Table 1). Median age was 28 years [interquartile range (IQR): 24, 33]. Any history of a sexually transmitted infection (STI) was reported by 46 (21%) women, 128 (57%) were HSV-2 seropositive, and 35 (17%) had bacterial vaginosis. Vaginal washing was reported by 61 (26%) women, and 116 (51%) reported using some method to make the vagina drier prior to sex. The CVS from 35 (17%) women had PSA of at least 1 ng/ml, indicating recent unprotected sexual activity. Couples reported a median of 6 sex acts (IQR: 2.5, 9) in the month before enrollment and 92 (40%) reported unprotected sex with their study partner in the last month. Among the women from discordant couples, the median plasma HIV-1 RNA level of their HIV-1 infected partners was 4.8 $\log_{10}$ copies/ml (IQR: 4.1, 5.3).

Sociodemographic and behavioral factors were similar between women who were using a form of hormonal contraception (DMPA, oral, or implantable) and those who were not using hormonal contraception; however, women who were not using hormonal contraception had an older median age than DMPA users (28 vs. 26 years, respectively). A larger proportion of women using implants were HSV-2 seropositive (83% in implant users vs. 57% in non-users and 53% among DMPA and oral contraceptive users). Women using oral contraception reported a higher median number of sex acts in the past month compared to other groups (11 vs. 5 sex acts per month, respectively), and women who were using DMPA or oral contraception were more likely to have PSA present in their CVS compared to women who were not using hormonal contraception (24% and 29% vs. 14%, respectively).

Levels of cationic polypeptides

Across all 228 study participants, of the cationic polypeptides measured in CVS samples, lactoferrin (2.72 $\log_{10}$ ng/ml) was found in the highest mean concentration, followed closely by HNP1–3 (2.13 $\log_{10}$ ng/ml). Levels of SLPI (1.92 $\log_{10}$ ng/ml) were lower, and LL-37 (0.51 $\log_{10}$ ng/ml) and HBD-2 (-0.11 $\log_{10}$ ng/ml) were found at the lowest levels. HBD-2 was undetectable in 56 (26%) women, LL-37 was undetectable in 42 (20%) of women, HNP1–3 was undetectable in 9 (4%) women, SLPI was undetectable in 5 (2%) women, and lactoferrin was undetectable in 3 (1%) women. There were significant correlations between HNP1–3, LL-37, lactoferrin, and HBD-2, and a weak correlation between lactoferrin and SLPI (Table 2). The strongest correlations were between lactoferrin and all other peptides. The correlation coefficient between lactoferrin and HNP1–3 was 0.43 (p<0.001), between lactoferrin and LL-37 was 0.39 (p<0.001), between lactoferrin and HBD-2 was 0.37 (p<0.001), and between lactoferrin and SLPI was 0.14 (p=0.040).

Correlates of cationic polypeptide levels

To identify potential confounding by sociodemographic, behavioral, and biological factors, we investigated correlates of cationic polypeptide concentrations in the study population. Compared to women from discordant couples, those from concordant negative couples had significantly higher mean concentrations of lactoferrin (3.08 vs. 2.57 $\log_{10}$ ng/ml; p<0.001) and HBD-2 (0.28 vs. −0.27 $\log_{10}$ ng/ml; p=0.005) (Table 3). There were no differences between these two groups of women for HNP1–3 (p=0.46), LL-37 (p=0.60), or SLPI (p=0.72). Older age was associated with lower levels of HBD-2 (p=0.004). The levels of HBD-2 were also higher among women who reported a desired for additional children (p=0.014). Unprotected sex in the past month was associated with higher levels of lactoferrin (p<0.001). There were trends indicating that the presence of PSA was associated with higher levels of SLPI (p=0.13) and lactoferrin (p=0.089), and with lower levels of LL-37 (p=0.11). These associations were unaffected by adjustment for contraceptive use (data not shown).

Hormonal contraceptive use and cationic peptide levels

In terms of associations between hormonal contraceptive use with cationic peptide levels, we found no evidence of effect modification by HIV exposure (i.e., HIV-discordant couple versus concordant negative couple), and therefore combined both groups of women in our evaluations of hormonal contraceptive use (electronic supplement 1). Compared to women not using hormonal contraception (n=165), DMPA users (n=41) had significantly higher mean levels of HNP1–3 (2.38 vs. 2.04 $\log_{10}$ ng/ml; p=0.024), LL-37 (0.81 vs. 0.40 $\log_{10}$ ng/ml; p=0.027), and lactoferrin (3.03 vs. 2.60 $\log_{10}$ ng/ml; p=0.002) (Figure 1). These associations were not affected by statistical adjustment for potential confounders (presence of PSA, frequency of sex in the past month, and discordant vs. concordant negative couple) (Table 4). There were no associations between DMPA and the levels of SLPI (p=0.27) or HBD-2 (p=0.43), relative to women not using hormonal contraception.

Compared to women not using hormonal contraception, oral contraceptive use (n=16) was not associated with HNP1–3 (p=0.40), LL-37 (p=0.53), or HBD-2 (p=0.18) but there was a trend indicating an association with lactoferrin (p=0.12) (Table 4). Unlike DMPA users,

women using oral contraceptives had higher levels of SLPI compared to women not using hormonal contraception, nearly reaching statistical significance (2.27 vs. 1.86 $\log_{10}$ ng/ml; p=0.054).

Women using contraceptive implants (n=6) had significantly higher levels of LL-37 (1.23 vs. 0.40 $\log_{10}$ ng/ml; p=0.040) and lactoferrin (3.34 vs. 2.60 $\log_{10}$ ng/ml; p=0.02), compared to women not using hormonal contraception. There was also a trend indicating implant users had higher levels of HNP1–3 (2.63 vs. 2.04 $\log_{10}$ ng/ml; p=0.087). The point estimates for the difference between implant users and women who were not using hormonal contraception were larger than the corresponding estimates for DMPA users.

Discussion

We found that women who were using DMPA had significantly higher levels of the cationic polypeptides HNP1–3, LL-37, and lactoferrin in their CVS than women not using hormonal contraception. These regulators of the innate immune response in the female genital mucosa may contribute to an increased risk of HIV-1 infection associated with DMPA use. Previous studies have found that DMPA users have an elevated risk of HIV-1 infection;[4,32–36] however, not all studies have confirmed this association,[3] and those that have may be subject to residual confounding by sexual risk behavior. Therefore, it is important to investigate potential mechanisms to separate the biological and behavioral pathways leading to elevated risk. The use of DMPA involves a large dose of a synthetic progestin administered every three months. While highly effective in preventing pregnancy, the absence of estrogen and persistently high progestin levels induce considerable changes in the genital mucosa. Mechanisms proposed for the increased biological HIV-1 risk associated with DMPA fall into three categories: (1) Changes in vaginal and cervical structure, including cervical ectopy and vaginal thinning, (2) changes in local or systemic immunity, and (3) increased risk of other sexually transmitted infections and detrimental changes in the vaginal flora.[21] Any or all of these factors may play a role, but the findings from this study provide increasing evidence that DMPA use has a substantial effect on the innate immune response in the female genital mucosa.

In the context of HIV-1 exposure, HNP1–3, LL-37, and lactoferrin may exert two opposing actions on the innate immune response, the balance of which determines their overall effect on HIV-1 infection risk. While HNP1–3, LL-37, and lactoferrin are all known to have intrinsic antiviral properties (potentially decrease infection risk),[8,37–39] HNP1–3 and LL-37 are also potent recruiters of immune cells that are susceptible to HIV-1 infection (potentially increase infection risk).[40] Increased LL-37 production has also been shown to enhance HIV-1 transmission by up-regulating HIV co-receptors on Langerhans cells in human skin explants,[41] and the active component of DMPA prevents the down-regulation of HIV co-receptors on T cells after activation.[19] In agreement with this *in vitro* evidence, elevated levels of HNP1–3 and LL-37 in CVS have been associated with increased HIV infection risk,[12] and DMPA use has been shown to increase numbers of potential HIV target cells and cells expressing activation markers in the human vagina.[42] Thus, the observed increase in the production of some cationic polypeptides in the female genital mucosa of DMPA users

may provide a mechanistic explanation for an environment that favors mucosal transmission of HIV-1.[43]

Correlation analysis of the levels of cationic polypeptides in CVS showed that HNP1–3 and LL-37 exhibit the strongest correlation among the five analyzed peptides, consistent with previous findings.[44] We also detected a strong correlation between lactoferrin and HNP1–3 as well as between lactoferrin and LL-37. CVS components stem from multiple locations across the female genital tract and are most likely produced by a variety of cell types, upon different stimuli. Nevertheless, the strong correlation observed between HNP1–3, LL-37, and lactoferrin in CVS points towards neutrophils as major producers of these peptides in the female genital mucosa,[45] and therefore a potential target of DMPA regulatory effect. *In situ* analysis of the expression of the three peptides in the mucosa from the upper and lower female genital tract may help to corroborate this hypothesis.

Oral contraceptive use, which involves administration of low doses of estrogen and progesterone analogues, appears to have a qualitatively different relationship with cationic peptide levels compared to DMPA use. Unlike DMPA users, there was little or no evidence that oral contraceptive users differed from non-users of hormonal contraception in their levels of HNP1–3 or LL-37. However, oral contraceptive users had higher levels of SLPI while no such association was found among DMPA users. DMPA and oral contraceptive users were more similar in their elevated levels of lactoferrin compared to non-users of hormonal contraception. In contrast, women using progestogen-based implantable contraception had even higher levels of HNP1–3, LL-37, and lactoferrin compared to DMPA users; however, this was based on a small number of women using implantable contraception (n=6) and it was not possible to adjust for potentially confounding factors. Given the rare but increasing usage of contraceptive implants, more research is needed into their safety in areas of high HIV-1 incidence.

Interestingly, we also found weak evidence that recent unprotected sex, indicated by the presence of PSA in the CVS sample, was associated with lower levels of HNP1–3 and LL-37, and with higher levels of lactoferrin. Significantly higher lactoferrin levels were also found in women who reported any unprotected sex in the past month. The lower concentrations of some of these polypeptides may be due to the immunoregulatory effect of semen in the female genital mucosa.[46,47] These observations further emphasize the complex nature of the innate immune response in the genital mucosa, and indicate that the overall effect on HIV-1 risk may be the sum of all factors influencing the local environment at the time of viral exposure.

This observational study adds to existing evidence that DMPA is associated with biological effects that may predispose a woman to HIV-1 infection. However, this cohort experienced a low rate of HIV-1 transmission, with 4 seroconversions (1.9 per 100 woman-years) among women not using hormonal contraception, 2 seroconversions (22.8 per 100 woman-years) among implant users and no seroconversions among DMPA or oral contraceptive users (data not shown). While this study was not designed to evaluate the safety of DMPA, it illustrates that very low rates of HIV-1 transmission can be achieved among high-risk women using DMPA, which we attribute to access to good clinical and counseling resources; this is

especially notable since the baseline risk of HIV-1 infection was considerably elevated as all women followed longitudinally had HIV-positive partners. The resources available to women in this study included comprehensive diagnosis and treatment of STIs for both them and their partners, access to unlimited condoms and continued counseling about use of condoms in serodiscordant couples, and access to family planning services. As recent WHO recommendations indicate, the choice of contraceptive methods must be considered against a complex interaction of factors including fertility desire, relationship dynamics, reproductive health, and HIV-1 risk.[48] There is a considerable need to improve the available options for contraception, particularly in sub-Saharan Africa, but in the near future DMPA will continue to be an important component of the method mix. Appropriate resources are needed to meet women's contraceptive needs while minimizing their risk of both HIV-1 infection and unintended pregnancies. Expansion of antiretroviral therapy, including 'treatment as prevention,' could make DMPA safer among women at increased risk of HIV acquisition.

This was a relatively large study of HIV-1-uninfected women, including those who were and were not exposed to HIV-1 by an infected male partner. Samples of CVS were collected by swab under consistent conditions in the context of a larger cohort study. Despite these strengths, some limitations exist that affect our interpretation of these data. Hormonal contraceptive use was self-reported. Any resulting misclassification is unlikely to be related to peptide levels, or correlates of peptide levels, and therefore the effect is likely an underestimate of the true associations. The process of collecting cervicovaginal swabs is subject to some variability that decreases the precision of the peptide measurements. Furthermore, we were unable to account for variations in peptide levels throughout the menstrual cycle. Again, the likely effect would be an underestimate of associations. We found some evidence that not all forms of hormonal contraception (e.g., DMPA, oral contraception, and implantable contraception) have the same associations with innate polypeptide peptide levels, but the relatively small number of women who were using oral and implantable contraception limited our ability to explore these differences. We could not distinguish endogenous peptides from peptides originating from semen; however, recent unprotected sex, as measured by the presence of PSA, was associated with lower levels of HNP1–3 and LL-37, indicating these peptides were unlikely to originate from semen. Furthermore, statistical adjustment for the presence of PSA did not affect the observed associations. Finally, we were unable to directly assess the effect of innate peptide levels on HIV-1 risk due to the very low rate of HIV-1 transmission in this cohort.[22]

Conclusions

We found that DMPA use was associated with higher concentrations of cationic polypeptides in the female genital mucosa. Some of these peptides have been found previously to recruit immune cells and are associated with increased risk of HIV-1 infection. Progestin-based contraception may upregulate these polypeptides in the female genital mucosa, which could result in local recruitment of cells that are susceptible to HIV infection. This may provide independent evidence supporting a biological mechanism for increased HIV-1 acquisition seen with DMPA.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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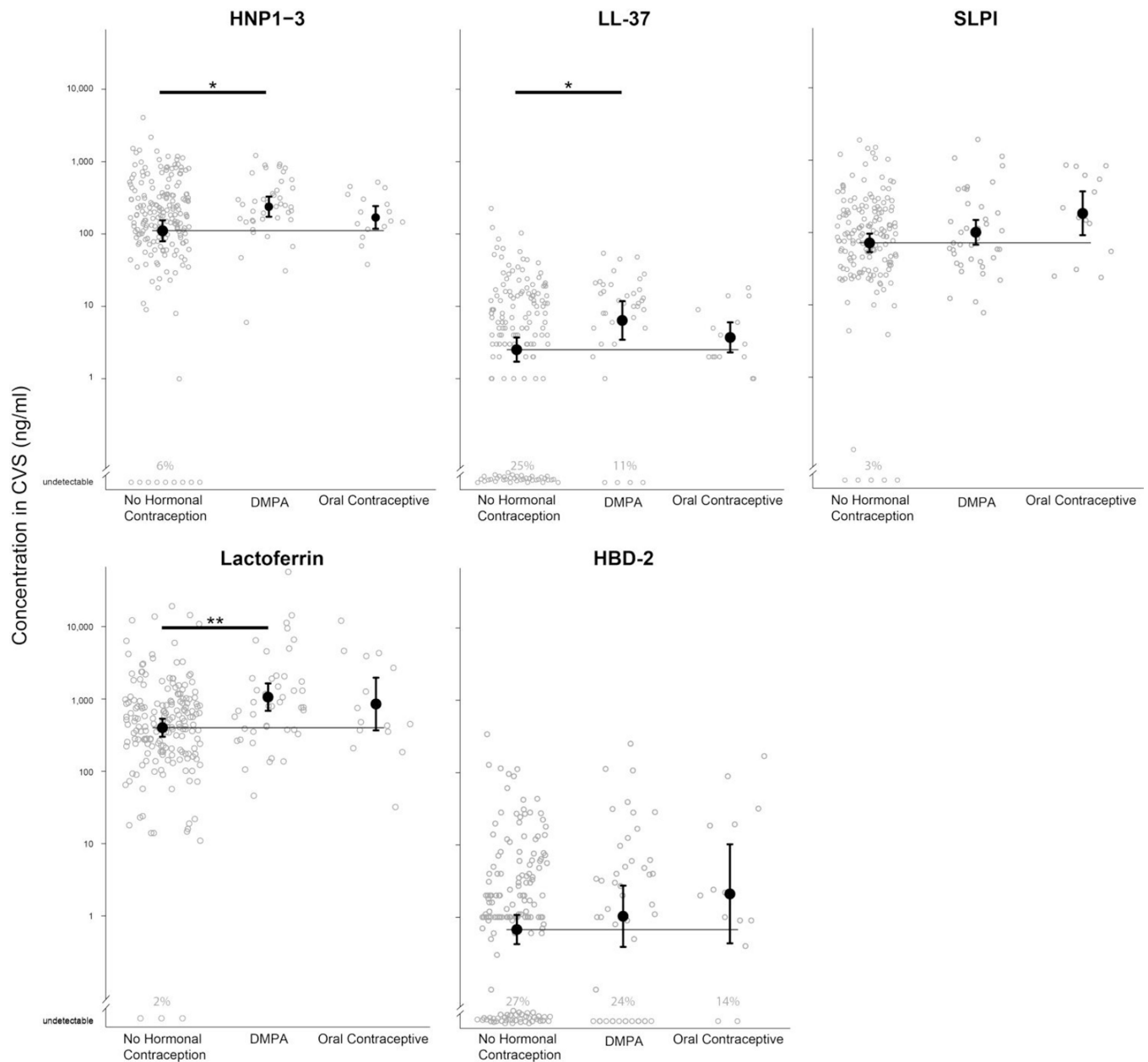
Figure 1.
Mean concentrations of cationic peptides in cervicovaginal sections (CVS) of HIV-1-uninfected women, stratified by hormonal contraceptive use. Concentrations are expressed as ng/ml. Error bars indicate the 95% confidence interval for the mean estimate. Thin horizontal lines indicate the mean concentration in women using no hormonal contraception. Bold horizontal lines indicate statistical significance at p < 0.05 (*) and p < 0.01 (**). Individuals with peptide levels below the lower limit of detection are indicated at the bottom of each plot. The lower limit of detection for HNP1–3 was 0.156 ng/ml, for LL-37 was 0.100 ng/ml, for SLPI was 0.0625 ng/ml, for lactoferrin was 0.400 ng/ml, and for HBD-2 was 0.0156 ng/ml.

Table 1

Demographic and behavioral characteristics of study participants, by hormonal contraceptive use.

| | Hormonal Contraception | | | |
	None N=165	DMPA N=41	Oral N=16	Implantable N=6
	n (%) or median (IQR)			
Age	28 (24, 34)	26 (24, 30)	27.5 (23, 32.5)	29 (24, 38)
Sex acts in past month	5 (2.5, 8.5)	5 (2.5, 8)	11 (6, 12.25)	5 (2, 11.5)
Any unprotected sex	64 (38.8)	16 (39.0)	12 (75.0)	0 (0)
Vaginal washing	40 (24.2)	16 (39.0)	3 (18.8)	2 (33.3)
Vaginal drying	77 (46.7)	25 (61.0)	11 (68.8)	3 (50.0)
Lifetime STI	38 (24.1)	6 (15.0)	2 (12.5)	0 (0)
HSV-2 seropositive	94 (57.3)	21 (52.5)	8 (53.3)	5 (83.3)
Bacterial vaginosis	27 (17.1)	7 (20.0)	0 (0)	1 (16.7)
Vaginitis	11 (6.9)	0 (0)	0 (0)	0 (0)
Cervicitis	2 (1.3)	0 (0)	0 (0)	0 (0)
Syphilis	1 (0.6)	0 (0)	0 (0)	0 (0)
Trichomoniasis	5 (3.3)	2 (5.7)	0 (0)	0 (0)
Desire additional children	87 (52.7)	23 (56.1)	12 (75.0)	1 (16.7)
Partner viral load ($\log_{10}$ copies/ml)	4.8 (4.0, 5.4)	4.6 (4.1, 4.9)	5.5 (5.2, 5.6)	5.2 (4.3, 5.3)
PSA present	22 (14.3)	9 (23.7)	4 (28.6)	0 (0)
HIV-discordant relationship	122 (73.9)	25 (61.1)	6 (37.5)	5 (83.3)

Abbreviations: DMPA, depot medroxyprogesterone acetate; IQR, interquartile range; STI, sexually transmitted infection.

Table 2

The relationship between the levels of cationic polypeptides within individuals is shown in the matrix of Pearson's correlation coefficients. Values closer to 0 indicate a lack of correlation and values closer to 1 indicate polypeptide levels are more highly correlated within individuals, and a negative coefficient indicates inverse correlation.

	HNP1–3	LL-37	SLPI	Lactoferrin	HBD-2
HNP1–3	1				
LL-37	0.50**	1			
SLPI	0.06	−0.06	1		
Lactoferrin	0.43**	0.39**	0.14*	1	
HBD-2	0.19**	0.15*	0.10	0.37**	1

Abbreviations: HNP1–3, human neutrophil peptides 1–3; SLPI, secretory leukocyte protease inhibitor; HBD-2, human beta-defensin 2

[a]Log10 transformed

*
p<0.05

**
p<0.01

Table 3

Correlates of cationic peptide concentration in cervicovaginal secretions.

	HNP1-3 Mean (SD)	Diff.	p	LL-37 Mean (SD)	Diff.	p	SLPI Mean (SD)	Diff.	p	Lactoferrin Mean (SD)	Diff.	P	HBD-2 Mean (SD)	Diff.	p
Couple HIV status															
Concordant negative	2.20 (0.75)	ref		0.45 (0.93)	ref		1.95 (1.01)	ref		3.08 (0.74)	ref		0.28 (1.20)	ref	
Discordant	2.11 (0.86)	−0.09	0.46	0.53 (1.03)	0.08	0.60	1.91 (0.63)	−0.04	0.72	**2.57 (0.51)**	**−0.50**	**<0.001****	**−0.27 (1.33)**	**−0.55**	**0.005****
Age	–	−0.003	0.70	–	−0.02	0.10	–	−0.007	0.31	–	−0.008	0.22	–	**−0.04**	**0.004****
Lifetime STI															
no	2.16 (0.80)	ref		0.58 (0.93)	ref		1.90 (0.79)	ref		2.74 (0.82)	ref		−0.09 (1.34)	ref	
yes	2.05 (0.97)	−0.11	0.43	0.36 (1.15)	−0.22	0.19	1.94 (0.68)	0.05	0.71	2.69 (0.61)	−0.05	0.68	−0.19 (1.26)	−0.10	0.65
HSV-2															
negative	2.14 (0.82)	ref		0.53 (0.97)	ref		1.87 (0.96)	ref		2.73 (0.93)	ref		−0.17 (1.37)	ref	
equivocal	2.41 (0.33)	0.27	0.26	0.95 (0.73)	0.42	0.14	2.00 (0.54)	0.13	0.52	2.83 (0.62)	0.10	0.62	0.37 (1.24)	0.54	0.12
positive	2.09 (0.88)	−0.05	0.67	0.43 (1.04)	−0.10	0.50	1.92 (0.67)	0.05	0.69	2.68 (0.69)	−0.04	0.72	−0.13 (1.28)	0.04	0.83
Bacterial vaginosis															
no	2.15 (0.78)	ref		0.51 (1.01)	ref		1.93 (0.74)	ref		2.66 (0.78)	ref		−0.12 (1.29)	ref	
yes	1.91 (1.15)	−0.24	0.15	0.41 (1.05)	−0.09	0.64	1.85 (0.74)	−0.07	0.59	2.78 (0.65)	0.11	0.43	−0.35 (1.34)	−0.23	0.35
Treatable genital infection															
no	2.11 (0.80)	ref		0.49 (1.01)	ref		1.93 (0.76)	ref		2.63 (0.79)	ref		−0.17 (1.27)	ref	
yes	2.06 (1.05)	−0.06	0.721	0.56 (1.05)	0.07	0.686	1.88 (0.70)	−0.05	0.687	2.87 (0.62)	0.24	0.062	−0.02 (1.35)	0.16	0.48
Sex acts in past month	–	−0.006	0.58	–	−0.02	0.12	–	**0.02**	**0.016***	–	−0.005	0.61	–	0.03	0.076
Any unprotected sex															
no	2.10 (0.86)	ref		0.51 (1.05)	ref		1.85 (0.71)	ref		2.53 (0.82)	ref		−0.25 (1.30)	ref	
yes	2.20 (0.77)	0.10	0.39	0.50 (0.92)	−0.01	0.93	2.01 (0.83)	0.16	0.12	**3.00 (0.63)**	**0.47**	**<0.001****	0.10 (1.05)	0.35	0.055
Vaginal washing															
no	2.14 (0.81)	ref		0.50 (1.01)	ref		1.89 (0.77)	ref		2.74 (0.79)	ref		−0.06 (1.31)	ref	

	HNP1–3 Mean (SD)	Diff.	p	LL-37 Mean (SD)	Diff.	p	SLPI Mean (SD)	Diff.	p	Lactoferrin Mean (SD)	Diff.	P	HBD-2 Mean (SD)	Diff.	p
yes	2.11 (0.88)	−0.03	0.81	0.52 (0.98)	0.01	0.94	1.98 (0.73)	0.09	0.45	2.70 (0.76)	−0.07	0.54	−0.24 (1.34)	−0.18	0.38
Vaginal drying															
no	2.14 (0.79)	ref		0.61 (0.91)	ref		1.87 (0.66)	ref		2.71 (0.91)	ref		−0.11 (1.04)	ref	
yes	2.13 (0.87)	−0.01	0.90	0.40 (1.08)	−0.20	0.14	1.96 (0.86)	0.08	0.40	2.73 (0.62)	0.02	0.88	−0.10 (0.99)	0.01	0.97
Desire additional children															
no	2.20 (0.73)	ref		0.51 (1.02)	ref		1.91 (0.76)	ref		2.69 (0.66)	ref		−0.34 (1.32)	ref	
yes	2.08 (0.90)	−0.11	0.31	0.51 (0.98)	0.002	0.99	1.92 (0.77)	0.008	0.94	2.75 (0.87)	0.06	0.58	**0.09 (1.29)**	**0.43**	**0.015**[*]
Partner's viral load ($\log_{10}$ copies/ml)	–	0.07	0.20	–	**0.14**	**0.040**[*]	–	−0.005	0.90	–	0.005	0.92	–	0.03	0.75
PSA present															
no	2.16 (0.82)	ref		0.53 (1.02)	ref		1.89 (0.76)	ref		2.68 (0.81)	ref		−0.13 (1.31)	ref	
yes	1.94 (0.96)	−0.22	0.17	0.23 (0.99)	−0.302	0.11	2.10 (0.63)	0.21	0.13	2.94 (0.70)	0.26	0.089	−0.10 (1.41)	0.03	0.92

Abbreviations: HIV, human immunodeficiency virus; HSV, herpes simplex virus; PSA, prostate specific antigen; HNP1–3, human neutrophil peptides 1–3; SLPI, secretory leukocyte protease inhibitor; HBD-2, human beta-defensin 2; SD, standard deviation; NA, not applicable

NOTE – Polypeptide concentrations are expressed as $\log_{10}$ ng/ml; Treatable genital infections included bacterial vaginosis, trichomoniasis, syphilis, cervicitis, and vaginitis

[*]
p<0.05

[**]
p<0.01

Table 4

Cationic polypeptide levels in cervicovaginal secretions, by hormonal contraceptive use.

	No Hormonal Mean (SD)	DMPA Mean (SD)	Diff.	p	Adj. Diff.[a]	Adj. p	Oral Mean (SD)	Diff.	p	Adj. Diff.[a]	Adj. p
HNP1–3	2.04 (0.92)	**2.38 (0.44)**	0.33	0.024[*]	0.33	0.036[*]	2.23 (0.31)	0.19	0.40	0.17	0.49
LL-37	0.40 (1.06)	**0.81 (0.83)**	0.40	0.027[*]	0.43	0.027[*]	0.57 (0.41)	0.17	0.53	0.28	0.35
SLPI	1.86 (0.82)	2.00 (0.56)	0.15	0.27	0.12	0.37	2.27 (0.58)	0.41	0.054	0.36	0.12
Lactoferrin	2.60 (0.80)	**3.03 (0.62)**	0.42	0.002[**]	0.36	0.010[*]	2.93 (0.70)	0.33	0.12	0.27	0.26
HBD-2	−0.17 (1.30)	0.01 (1.38)	0.18	0.43	0.27	0.26	0.33 (1.31)	0.49	0.18	0.10	0.81

Abbreviations: DMPA, depot medroxyprogesterone acetate; HNP1–3, human neutrophil peptides 1–3; SLPI, secretory leukocyte protease inhibitor; HBD-2, human beta-defensin 2; SD, standard deviation; Diff, difference, compared to women using no hormonal contraception; Adj, adjusted value

NOTE – Polypeptide concentrations are expressed as log10 ng/ml

[a] Adjusted for presence of PSA in CVS sample, frequency of sex in the past month, and HIV exposure group (i.e., discordant versus concordant negative couple)

[*] p<0.05

[**] p<0.01