

Title: Mechanisms of HIV transmission in Depo-Provera users: The likely role of hypoestrogenism

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Abstract

Almost half of new HIV infections worldwide occur in women, and vaginal intercourse is the most common mode of transmission. Accumulating evidence suggests that depot medroxyprogesterone acetate (DMPA, Depo-Provera) may increase HIV transmission, but little is known about the underlying mechanisms. We propose that hypoestrogenism in DMPA may contribute to increased HIV transmission. We present supportive evidence and propose potential interventions to prevent or treat vaginal hypoestrogenism using vaginal estrogens.

Background

HIV infects around 2.3 million people annually, almost half of whom are women ¹. Worldwide, vaginal intercourse is the most common mode of transmission, accounting for more than 80% of new female cases ². In sub-Saharan Africa, women are infected 5 to 7 years younger than men ².

Promotion of safe and effective contraception is central to the well-being and autonomy of women, and saves millions of lives worldwide by reducing maternal morbidity and mortality, and by preventing unplanned pregnancy and consequent infant mortality ³. Effective contraception is particularly critical in areas of high HIV prevalence in order to prevent vertical transmission, because Prevention of Mother-to-Child Transmission (PMTCT) interventions have low coverage in resource-poor countries ⁴.

Injectable progestins are among the most common hormonal contraceptive methods, used by around 47 million women worldwide ⁵ of whom around 9 million are in Southern and Eastern Africa. Available products are depot medroxyprogesterone acetate (DMPA, Depo-Provera) and norethisterone enanthate (NET-EN). DMPA is much more widely used and has the advantage of 12-weekly administration (versus 4-weekly for NET-EN). Depo-Provera is popular amongst women, health care providers, rural health workers and international agencies due to low cost, safety, effectiveness, prolonged duration of action, reversibility, ease of use, privacy, and convenience.

In primate models, DMPA substantially enhances vaginal simian immunodeficiency virus (SIV) acquisition ^{6,7}. Although human clinical studies have been inconsistent and there have been no adequately powered randomized controlled trials ³, there is

burgeoning evidence that DMPA may increase both acquisition and transmission of HIV^{3, 8-10}. Despite limited data, existing evidence suggests that neither combined oral contraceptives (COC) nor NET-EN increase HIV risk^{3, 10, 11}. Pooling ten studies, Ralph *et al.* found that DMPA increased risk of HIV acquisition (hazard ratio (HR) 1.40, 95% confidence interval (CI) 1.16-1.69), but COC did not (HR 1.00, 95% CI 0.86-1.16)¹⁰. In adjusted analysis of individual participant data from 18 studies, Morrison *et al.* also found that DMPA increased risk of HIV acquisition (HR 1.50, 95% CI 1.24-1.83) but that there was no statistically significant association between HIV acquisition and COC (HR 1.03, 95% CI 0.88-1.20) or NET-EN (HR 1.24, 95% CI 0.84-1.82)¹¹.

The possibility that a widely used contraceptive promoted in populations with high HIV prevalence may increase transmission is of urgent public health and clinical concern³. This could potentially be responsible for 27,000-130,000 additional infections *per annum* globally, nearly all in resource-poor areas¹². Limited contraceptive alternatives in these regions mean that avoidance of DMPA could increase unplanned pregnancy and maternal mortality. These concerns have prompted a review of guidelines on medical eligibility for contraceptive use by WHO^{13, 14} and CDC¹⁵ which concluded that there is continuing uncertainty about the relationship between DMPA and HIV acquisition, and a need for adequately powered randomized controlled trials to answer this question¹⁶. The ECHO Consortium (Evidence for Contraceptive Options and HIV Outcomes) is planning a three-armed comparison trial of around 9000 women randomized to DMPA, Jadelle (levonorgestrel contraceptive implant) and copper IUD in areas of high HIV prevalence¹⁶. Whilst there has been extensive debate about the feasibility and ethics of such a trial^{17, 18}, there is extensive support from local specialists and consumer advocates for definitive information to inform clinical practice, contraceptive access, and policy for women in high risk areas.

Hypoestrogenism and DMPA

The principal contraceptive action of DMPA is ovulation suppression. With continued use, circulating estradiol concentrations commonly fall into the postmenopausal range^{19, 20}. Of key significance is that this uncompensated hypoestrogenism appears to be unique to DMPA and does not occur with other hormonal contraceptives, either because they contain estrogens (e.g. combined hormonal contraceptives, contraceptive vaginal ring), do not completely suppress endogenous estradiol (e.g. progestin-only implants), or do not consistently suppress ovulation (e.g. levonorgestrel-containing IUDs)²¹. NET-EN has a similar mechanism of action to DMPA, but limited data from NET-EN users suggests that estradiol levels remain in the normal premenopausal range^{22, 23} (Figure 1). Systemic hypoestrogenism in DMPA users is related to body mass index (BMI), and estradiol concentrations show greater variability in obese women than normal weight women²⁴. If hypoestrogenism contributes to HIV transmission in DMPA users, this may be of greater significance to women in resource-poor countries where BMI is generally lower.

Lactational amenorrhea (LAM) is a natural family planning method used by up to 20% of women in areas of high HIV prevalence²⁵. During LAM, breast feeding suppresses ovulation via high circulating prolactin. Circulating estradiol concentrations are characteristically reduced during LAM, but women are not usually profoundly hypoestrogenic²⁶ with mean circulating estradiol concentrations of around 120 pmol/l in one prospective study²⁷. Little is known about the relationship between LAM and HIV transmission.

Mechanisms by which DMPA may promote HIV infection in the vagina

Mechanisms of increased HIV acquisition with DMPA and other hormonal contraceptives are currently the subject of intense investigation to provide biological plausibility to observational data ^{3, 10, 11}. Elucidating these mechanisms would also increase understanding of the impact of sex hormones on HIV sexual transmission, and may inform the selection of safer alternative hormonal contraceptives and the development of targeted multipurpose prevention strategies to prevent pregnancy and HIV transmission.

The prevailing view has been that HIV infection is established via entry through the relatively superficial single layer of columnar ectocervical epithelium and the cervical transformation zone separating the ectocervix and endocervix ²⁸. This is the most immunologically active site in the reproductive tract with a relative abundance of lymphocytes and antigen presenting cells ²⁹. However, recent human and experimental data suggest that sexual transmission of HIV in women can occur throughout the reproductive tract including the squamous ectocervical and vaginal mucosae ^{28, 30, 31}. Potential mechanisms by which DMPA may increase the risk of HIV transmission via vaginal intercourse include disrupting physical barriers, induction of mucosal inflammation, decrease in protective innate and adaptive soluble and cellular immune responses, activation and recruitment of HIV target cells, alterations in vaginal microbiota, and promotion of HIV replication and transcytosis through female reproductive tract epithelial cells ³²⁻³⁵. DMPA may also indirectly increase risk by changing sexual behavior or condom use, or by promoting genital herpes or other sexually transmitted infection ³⁶. The medroxyprogesterone (MPA) in DMPA binds with higher affinity to glucocorticoid receptor than do the norethindrone in NET-EN or

levonorgestrel in COC, ³⁷ and has immunosuppressive effects on T cells and plasmacytoid dendritic cells not seen with other exogenous progestins ^{34, 37, 38}.

Physical effects of hypoestrogenism in the vagina

The vagina is highly estrogen-sensitive and will be affected by a fall in circulating estradiol concentrations, although vaginal and circulating estrogen concentrations may differ. Numerous studies in postmenopausal women, in whom systemic (ovarian) estradiol production is very low, report that genitourinary symptoms of menopause include vaginal dryness, discomfort, pallor, and loss of rugae, sometimes accompanied by yellowish vaginal discharge. Objective assessments of hypoestrogenism include reduced acidity (increased pH), reduced superficial cellular maturity (Vaginal maturation index, VMI) and changes to microbiota, including reduction in lactobacilli ³⁹. However, little is known about how menopause affects vaginal epithelial thickness. A recent small study comparing vaginal wall thickness in biopsies from pre- and postmenopausal women with uterovaginal prolapse reported significantly **greater** vaginal wall thickness in postmenopausal women compared to premenopausal women, apparently due to a thicker muscular layer ⁴⁰. The authors also report substantial variation in thickness at different vaginal locations, suggesting that single biopsies may not be representative.

Whilst hypoestrogenism in DMPA users is well established, few studies have investigated whether this might influence HIV transmission. Physiological hypoestrogenism at menopause commonly results in vaginal hypoestrogenism ⁴¹, but little is known about how systemic hypoestrogenism in DMPA users impacts the female reproductive tract. DMPA has extensive effects in the vagina, but it is not known whether these are modulated via changes in systemic estrogen concentrations.

Further, the prevalence or nature of vaginal symptoms associated with hypoestrogenism have not routinely been collected in DMPA studies, although one recent study reports that vaginal dryness affects at least 10%⁴² and that a characteristic “postmenopausal” vaginal appearance may be seen⁴³. Vasomotor symptoms characteristic of menopause have not been reported in DMPA users, but this may be because DMPA is a moderately effective treatment for vasomotor symptoms⁴⁴.

Several small studies have measured vaginal epithelial thickness in DMPA users, most reporting little or no change^{19, 20, 45, 46}. This contrasts with findings in non-human primates showing that DMPA promotes marked thinning of vaginal epithelium. Administration of DMPA is an established method of increasing SIV/SHIV infection in primate models for experimental studies^{6, 7}. In addition to anatomical differences between primates and women, studies in primates may not have accounted for the modifying effect of BMI on hypoestrogenism associated with DMPA. Pigtail macaques, which have BMI similar to adult women, demonstrated only modest thinning of vaginal epithelium⁴⁷ on treatment with physiological DMPA concentrations.

Epithelial thinning is not the only potential mechanism by which DMPA could impair integrity of the female genital tract. Decreased vaginal epithelial integrity following DMPA use could allow HIV to migrate deeper into stratified vaginal epithelium and increase the probability of encountering target cells including dendritic cells (DCs) and CD4+ T cells^{48, 49}. However, data regarding the impact of DMPA on the density of intercellular junctional proteins in vaginal epithelium is inconsistent⁴⁹. Whether changes in vaginal epithelial integrity are observed following longer DMPA treatment requires further investigation. Although older age has been noted as a risk factor in vaginal transmission of HIV^{50, 51}, surprisingly, only one publication (a letter) has

addressed vaginal transmission risk in postmenopausal women ⁵². This study reports three cases of women infected with HIV following fewer than 6 episodes of vaginal intercourse, and suggests that postmenopausal women are at higher risk than premenopausal women. Since the majority of postmenopausal women in developed countries are sexually active ³⁹, the impact of hypoestrogenism at menopause on HIV transmission requires urgent evaluation in larger studies.

Immune modulatory effects of DMPA in the vagina

DMPA impairs vaginal immune defenses by a local reduction in the antimicrobial peptides human β -defensin-2 and -3 ^{46, 53}, which have anti-HIV-1 activity ⁵⁴. It also increases vaginal levels of RANTES, a chemokine which competes with HIV for binding to the CCR5 co-receptor but also could recruit HIV target cells such as CD4+ T cells, DCs and macrophages to the mucosa ^{35, 53}. Higher RANTES levels in cervical samples from African women were predictive of HIV seroconversion ⁵³. DMPA suppresses the expression of key regulators of cellular and humoral immunity ^{35, 55}. This includes interferon alpha from plasmacytoid DCs, interferon gamma from activated T cells ⁵⁵, and inflammatory cytokines and chemokines (e.g. MIP-1 α) ³⁴. Interferon epsilon, a type I interferon constitutively expressed by female reproductive tract epithelial cells in mice and human cell lines, was shown to be protective against herpes simplex virus type 2 and *Chlamydia muridarum* ⁵⁶. Interferon epsilon is hormonally regulated although whether DMPA suppresses interferon epsilon in women and if it has inhibitory activity against HIV remain to be determined.

DMPA promotes activation and recruitment of immune cells. In contrast to suppressing soluble immune mediators, DMPA significantly alters vaginal immune cell populations by increasing numbers of T cells, macrophages, and HLA-DR- and CCR5-positive

cells which may increase susceptibility to HIV infection ⁴⁹. Activated CD4+ T-cells expressing CCR5 are elevated in the cervix of healthy postmenopausal women compared to premenopausal women ⁵⁷. However, recent evidence with long-term (\geq 6 to 24 months) DMPA treatment does not support this potential mechanism for DMPA enhancement of HIV transmission ⁵⁵. Instead a decrease in CD3+ T lymphocyte numbers was observed which might compromise adaptive immune defenses against HIV infection ⁵⁵. Whilst there is mounting evidence that immune changes due to DMPA could promote HIV infection, the effects of progesterone and estrogen on immune function in the female reproductive tract are complex. Both may influence immune function implicated in HIV transmission with distinct effects in the upper and lower tract, and the immune modulatory effects of estrogen are concentration dependent ^{33, 58-60}. Further *in vitro* studies using primary cells and tissues, *in vivo* studies in relevant animal models, and longitudinal studies in women will be required to explain any influence of DMPA on immune function to increase HIV susceptibility.

Effects of DMPA on the vaginal microbiota

Changes in the vaginal microbiota with DMPA are similar to those in postmenopausal women. Specifically, DMPA reduces vaginal colonization with H₂O₂-producing lactobacilli ^{19, 55}. A recent study reported colonization with such lactobacilli decreased by 49% ($p=0.03$) after 12 months on DMPA ($n=32$) ⁵⁵. Some *Lactobacillus* species (e.g. *L. crispatus*) are anti-inflammatory in vaginal epithelium and dampen pro-inflammatory cytokine and chemokine responses elicited by Toll-like receptor agonists which mimic viral and bacterial components ⁶¹⁻⁶³ that could promote HIV infection by recruitment and activation of target cells. Vaginal lactobacilli contribute to local defenses against HIV infection ^{64, 65}. Apart from their immune modulatory effects,

lactobacilli produce lactic acid that acidifies the vagina⁶⁶ and potentially inactivates HIV⁶⁷ and bacteria associated with bacterial vaginosis (BV)⁶⁸, which promote HIV acquisition⁶⁴. BV is a common imbalance of vaginal flora marked by overgrowth of obligate anaerobic bacteria and depletion of lactobacilli^{69,70}. In contrast to studies^{55,60} showing that DMPA decreases vaginal lactobacilli, a recent systematic review reported that COC and DMPA reduce BV by 10-20 and 18-30%, respectively⁷¹. These findings were supported by a second systematic review and meta-analysis⁷². Estrogen in COC could increase glycogen and its breakdown products, an energy source for lactobacilli⁷³, and estrogen may also increase overgrowth of vaginal *Candida* species^{71,74}. While the role of progesterone in the decrease observed in the meta-analyses^{71,72} is not clear, it has been proposed that amenorrhea caused by progestin-only contraception could decrease exposure of BV-associated bacteria to iron, an essential energy source for these bacteria^{72,75}. Molecular studies using culture-independent methods based on 16S ribosomal RNA gene sequences, rather than Nugent scores, to characterize distinct *Lactobacillus* species with different vaginal activity⁷⁶ may provide better insight into the significance of vaginal microbiota changes mediated by DMPA.

Direct effects of DMPA on HIV replication

DMPA increases HIV replication in CD4+ T cells³⁴. Enhancement of *in vitro* CC5-tropic HIV infection and replication by MPA³⁴ suggests that this mechanism could play a role in enhancing HIV susceptibility at the vaginal mucosa. In contrast, progesterone and estradiol have little and no effect, respectively, on HIV replication³⁴. MPA could enhance HIV replication by two possible mechanisms. It could upregulate HIV transcription through binding of a MPA-glucocorticoid receptor complex to glucocorticoid response elements in the HIV long terminal repeat⁷⁷. Alternatively, MPA

could block the production of antiviral cytokines that downmodulate the CCR5 chemokine receptor required for HIV entry ³⁴. Physiological levels of MPA were recently shown to increase HIV transcytosis by primary reproductive tract epithelial cells, resulting in enhanced infection of CD4+ T cells *in vitro* ³⁵. While progesterone also increased HIV transcytosis, the effect of MPA was greater and estradiol had no effect ³⁵.

Treatment of vaginal hypoestrogenism with topical estrogens

In primate models, administration of estrogen, systemically or vaginally, protects against DMPA-induced SIV infection ^{58, 78, 79}, which may be attributable to increased thickness of vaginal epithelium ⁷⁹ or to protection of primary target immune cells ^{80, 81}. Vaginal estrogens are widely used by postmenopausal women and are an established, effective treatment for vaginal dryness and atrophic vaginitis secondary to hypoestrogenism ^{82, 83}. Available preparations contain estriol, estradiol or conjugated estrogen (Premarin) as creams, pessaries or a vaginal ring, and these are similarly effective in treating vaginal symptoms at menopause. Treatment regimens vary, but intermittent use (2-3 times weekly) reduces local inflammation, corrects vaginal pH, restores vaginal cytology to premenopausal levels and resolves vaginal dryness with minimal systemic absorption ^{84, 85}. In experimental models of menopause, topical estrogen increases vaginal epithelial thickness ⁸⁶ and promotes extracellular matrix remodeling to restore structural integrity ⁸⁷. Small studies in postmenopausal women show that vaginal estrogen increases synthesis of mature collagen, decreased degradative enzyme activity, and increases thickness of the vaginal wall ⁸⁸. Systemic absorption of vaginal estrogen at therapeutic doses to treat vaginal atrophy is minimal ⁸⁵. Critically, primate data demonstrate that DMPA increases SIV transmission but that this can be prevented by vaginal estrogen treatment (as estriol) without detectable

systemic effects ⁷⁹. Since DMPA acts principally through the hypothalamic-pituitary axis to inhibit ovulation, the addition of exogenous estrogens does not impair the contraceptive efficacy of DMPA ⁸⁹.

A trial of local (vaginal) replacement estrogens in DMPA users would elucidate the contribution of hypoestrogenism to HIV acquisition in this population. Improving our understanding of this potential contribution is important because it provides insight into mechanisms of vaginal HIV transmission and into HIV risk for sexually active postmenopausal women. Further, it would provide key information about whether other hormonal contraceptives which do not cause hypoestrogenism are likely to affect HIV transmission, which would inform practice and policy. If the trial proved successful, it could increase the safety of DMPA without compromising efficacy, thereby improving safe contraceptive options for women.

If HIV transmission in DMPA users is regulated by hypoestrogenism, then vaginal estrogens could be a simple method to reverse these vaginal changes and potentially protect against HIV. Vaginal estrogens are cheap, and stable at room temperature. Those containing estradiol (e.g. Vagifem®) cost around USD\$16 per month and estriol cream (e.g. Ovestin®) around \$10. The estradiol-releasing vaginal ring (Estring®) is more costly but can be left in situ. Currently, the lifetime cost in the US of treating one person infected with HIV is estimated at USD\$379,668 ⁹⁰. Although generic antiretroviral drug costs are much lower in sub-Saharan Africa due to special terms in international trade law, HIV treatment is still costly at \$747 per patient per year ⁹¹. If a sexually active woman uses DMPA for contraception for 30 years (from ages 15 to 45), thrice weekly vaginal estrogen would cost an additional \$4,000, the cost of five years' ART. Specifically manufactured products and similar trade arrangements could further reduce this cost.

Conclusion

Providing affordable, effective and accessible contraception to women in areas of high HIV prevalence is an international health priority. Planned randomized comparative trials of DMPA vs. other contraceptive options should include the collection of mechanistic data to further understand how DMPA may alter HIV risk. If hypoestrogenism with DMPA underlies this risk, vaginal estrogens could potentially be protective. This might contribute to maintaining contraceptive options for disadvantaged women at high risk of HIV with limited contraceptive options.

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Figure Legend

Figure 1. Mean circulating estradiol in (a) normal premenopausal contraceptive women (N=78)⁹²; (b-f) premenopausal women using progestin-only contraceptives (b: Mirena, N=86⁹³; c: Implanon, N=11⁹⁴; d: Jadelle, N=88⁹⁵; e: NET-EN, N=73²³; f: DMPA, N=31)⁹⁶; and normal postmenopausal women (g, N=1446)⁹⁷. Dashed bars indicate 95% confidence intervals and solid bars standard deviation.

