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Lower concentrations of chemotactic cytokines and soluble innate factors in the lower female genital tract associated with the use of injectable hormonal contraceptive

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ABSTRACT

Progesterone-based injectable hormonal contraceptives (HCs) potentially modulate genital barrier integrity and regulate the innate immune environment in the female genital tract, thereby enhancing the risk of STIs or HIV infection. We investigated the effects of injectable HC use on concentrations of inflammatory cytokines and other soluble factors associated with genital epithelial repair and integrity. The concentrations of 42 inflammatory, regulatory, adaptive growth factors and hematopoietic cytokines, five matrix metalloproteinases (MMPs), and four tissue inhibitors of metalloproteinases (TIMPs) were measured in cervicovaginal lavages (CVLs) from 64 HIV-negative women using injectable HCs and 64 control women not using any HCs, in a matched case–control study. There were no differences between groups in the prevalence of bacterial vaginosis (BV; Nugent score $\geq$7), or common sexually transmitted infections (STIs). In multivariate analyses adjusting for condom use, sex work status, marital status, BV and STIs, median concentrations of chemokines (eotaxin, MCP-1, MDC), adaptive cytokines (IL-15), growth factors (PDGF-AA) and a metalloproteinase (TIMP-2) were significantly lower in CVLs from women using injectable HCs than controls. In addition, the pro-inflammatory cytokine IL-12p40 and the chemokine fractalkine were less likely to have detectable levels in women using injectable HCs compared with those not using HCs. We conclude that injectable HC use was broadly associated with an immunosuppressive female genital tract innate immune profile. While the relationship between injectable HC use and STI or HIV risk is yet to be resolved, our data suggest that the effects of injectable HCs were similar in STI-positive and STI-negative participants.

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1. Introduction

Internationally, hormonal contraceptives (HCs) are widely used by women to prevent unplanned pregnancies. In South Africa, more than half of women aged 15–49 years old use depot medroxyprogesterone acetate (DMPA) or norethisterone enanthate (Net-EN), with more than three times the number of women using DMPA than Net-EN (Sibeko et al., 2011; Department of Health, 2007). DMPA is an aqueous microcrystalline suspension (150 mg/ml) administered by intramuscular depot injection every three months, while NET-EN injection contains 200 mg/ml of norethisterone, which is effective for two months as contraception (Fraser and Weisberg, 1981). DMPA primarily provides contraceptive protection by suppressing natural cyclic fluctuations of female sex hormones, resulting in a hypo-estrogenic state (Jeppsson et al., 1982). In addition, DMPA induces atrophy of the endometrium by decreasing the glycogen content needed to provide energy for the development of the blastocyst after the morula has entered the uterine cavity (Hatcher et al., 2011; Mishell et al., 1968). Less is known about the endogenous effects of Net-EN.

High-dose DMPA use is common in the simian immunodeficiency virus (SIV) vaginal challenge models because it results in thinning of the vaginal epithelium, which enhances genital SIV infection (Abel et al., 2004; Wieser et al., 2001). The role of DMPA in increasing the risk of HIV infection is contentious. While some studies have reported such risks (Morrison et al., 2010; Ungchusak et al., 1996; Baeten et al., 2007; Kumwenda et al., 2008), others have found no association (Kleinschmidt et al., 2007; Reid et al., 2010). The impact of DMPA on genital epithelial barrier integrity is similarly contentious (Kiddugavu et al., 2003; Myer et al., 2007).

In addition to potentially increasing HIV acquisition risk, DMPA has been associated with an increased risk of *Chlamydia trachomatis* infection and a decreased risk of acquiring other sexually transmitted infections (STIs) such as *Trichomonas vaginalis* and bacterial vaginosis (BV) (Baeten et al., 2001; van de Wijgert et al., 2013).

It has been hypothesised that DMPA might increase HIV acquisition risks by changing the inflammatory or chemotactic environment of the genital mucosa so as to increase the recruitment of HIV-susceptible immune cells to the mucosa (Ildgruben et al., 2003; Miller et al., 2000; Wieser et al., 2001; Wira and Veronese, 2011). However, treatment of PBMC with DMPA has been shown to cause reduced production of several inflammatory and adaptive cytokines (Huijbregts et al., 2013). At the female genital mucosa, suppression of innate immune responses may influence susceptibility to infections. Moreover, matrix metalloproteinases (MMPs), which are required during normal reproductive processes (such as menstruation) for extracellular matrix degradation and tissue remodelling in the endometrial compartment of the upper genital tract (Lockwood and Schatz, 1996; Rodgers et al., 1993; Birkedal-Hansen, 1995), may influence epithelial barrier repair in the lower genital tract. MMPs are regulated by specific tissue inhibitors of metalloproteinases (TIMPs) (Fernandez-Catalan et al., 1998; Gomis-Ruth et al., 1997), which may similarly be involved in the maintenance of the lower reproductive tract barrier.

Defining the impact of injectable HCs on female genital tract innate immunity in relation to susceptibility to STIs or BV, will provide important insights into biological co-factors influencing HIV risk in women. The aim of this study was to compare concentrations of genital tract-soluble immune mediators (including cytokines, MMPs and TIMPs) between women using long-acting injectable HCs and women not using HCs, while accounting for BV and common STIs.

2. Materials and methods

2.1. Study design, participants and sample collection

Our study included 64 HIV-uninfected women using injectable HCs (DMPA or Net-EN) and 64 women not using HCs, enrolled into the prospective CAPRISA 002 observational cohort study of acute HIV infection conducted at the Centre for the AIDS Programme of Research in South Africa (CAPRISA), in Durban, KwaZulu-Natal Province, South Africa, as previously described (Mlisana et al., 2012; van Loggerenberg et al., 2012). Non-HC users were matched to injectable HC users based on age (within 5 years of age) at a 1:1 ratio. Demographic and clinical data were collected at enrolment using a structured questionnaire administered by a trained counsellor. Although data on type of contraception (injectable HCs, combined oral contraceptives (COCs), intrauterine devices (IUDs), condoms, diaphragms, foam and jelly, or sterilisation) were collected no information was collected on whether the injectable contraceptive being used was DMPA or Net-EN. We therefore report on injectable HCs in this study with a combination of DMPA and Net-EN users. Women using COC or any other form of hormonal contraception were excluded from the study, with the exception of IUD users. Laboratory samples, including cervicovaginal lavages (CVLs), were collected from each participant at enrolment by gently flushing the cervix and the lateral vaginal walls with 10 ml of sterile normal saline, as previously described by Mlisana et al. (2012). Volume of saline recovered after the lavage was not typically recorded. CVLs were transported within 4 h on ice from the site to the laboratory. In the laboratory, CVLs were centrifuged and the supernatant collected and stored at −80 °C. The protocol for this study was approved by the Ethics Review Committees of the University of KwaZulu-Natal and the University of Cape Town.

2.2. Testing for STIs and BV

At enrolment, vulvovaginal swabs collected from the posterior fornices and lateral vaginal walls from each woman were tested for *C. trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, HSV-2 reactivation and *T. vaginalis* using PCR. Gram staining was performed to diagnose BV using Nugent score ≥7 (Mlisana et al., 2012).