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Effect of progestins on immunity: medroxyprogesterone but not norethisterone or levonorgestrel suppresses the function of T cells and pDCs☆,☆☆,★

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Abstract

Objectives: The potential effect of hormonal contraception on HIV-1 acquisition and transmission represents an important public health issue. Several observational studies have suggested an association between the use of hormonal contraception, in particular injectable depot medroxyprogesterone acetate (DMPA), and an increased risk of HIV-1 acquisition and transmission. We and others have previously demonstrated that DMPA acts as a potent inhibitor of innate and adaptive immune mechanisms. The study presented here addresses the immunomodulatory properties of several common progestins with a potential to replace DMPA.
Study design: To identify safe alternatives to DMPA, we tested the effect of commonly used progestins on the function of human primary T cells and plasmacytoid dendritic cells (pDCs) obtained from the blood of healthy premenopausal women.
Results: Medroxyprogesterone acetate (MPA) inhibited the activation of T cells and pDCs in response to T cell receptor- and Toll-like receptor-mediated activation at physiological concentrations. Etonogestrel exerted a partial suppressive activity at high concentrations. In sharp contrast, norethisterone (NET) and levonorgestrel (LNG) did not exhibit detectable immunosuppressive activity.
Conclusion: Evidence indicating the immunosuppressive properties of DMPA strongly suggests that DMPA should be discontinued and replaced with other forms of long-term contraception. Since NET and LNG do not exert immunosuppressive properties at physiological concentrations, these progestins should be considered as alternative contraceptives for women at high risk for HIV-1 infection.
Implications: The presented data suggest that, at physiological levels, the progestins NET and LNG do not suppress cytokine production by immune cells and should be considered as alternatives to DMPA; however, more in vivo testing is needed to confirm this data.

Keywords: Hormonal contraception; HIV-1; AIDS; Progestins; DMPA; NET; LNG; ETG

1. Introduction

Contraception represents a critical component of preventive health care. It provides women with a control over their reproductive health, reduces the number of unintended pregnancies, decreases maternal and infant mortality and morbidity, reduces recourse to abortion, lowers the risk of mother-to child transmission of HIV-1, and provides additional benefits including reduction of poverty and improved access to education. The use of injectable hormonal contraceptives is highly popular as it provides multiple advantages over other forms of contraception including high effectiveness and a long-term effect [1,2]. Depot medroxyprogesterone acetate (DMPA; Depo-Provera), a progestin-only contraceptive typically administered in the form of a 3-monthly intramuscular injection, is one of the most commonly used contraceptives in sub-Saharan Africa and other areas with high HIV-1 prevalence [1,2]. It is estimated

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that 20–50 million women worldwide use DMPA and the number is steadily increasing [1,3,4]. In some countries, DMPA is the method of choice for over 50% of women using modern methods of contraception [1,2]. Unfortunately, multiple observational studies suggest an association between the use of hormonal contraception and increased risk of HIV-1 acquisition and transmission [5–12]. In most studies, the adjusted hazard ratio of HIV-1 acquisition associated with the use of DMPA is higher than that linked to the use of oral contraceptives or other forms of contraception [7–13]. Many studies do not distinguish between the two most common forms of injectable contraception, DMPA and norethisterone enanthate (NET-EN), and little attention has been placed on differential pharmacological and biological effects of these two contraceptives. Recent re-analysis of data by Heffron et al. showed that, within the injectable users, women outside of South Africa (consistent with DMPA usage) had higher HIV-1 risk (adjusted HR=3.9) than women living in South Africa where NET-EN is used more widely [13]. None of the three studies that specifically assessed the effect of NET-EN found a significant association with HIV-1 acquisition [3,14,15]; however, more data is urgently needed. Non-human primate studies demonstrate that DMPA enhances the risk of SIV acquisition via vaginal exposure and suggest that DMPA increases viral levels in the acute phase of infection and reduces the protective effect of prior immunization [16–19]. Recently, we have demonstrated that MPA suppresses the production of key T cell-derived regulators of cellular and humoral immunity involved in the induction of immune response to invading pathogens including interferon γ (IFN) γ, interleukin (IL)-2, IL-4, IL-6, IL-12, macrophage inflammatory protein (MIP)-1α, and tumor necrosis factor α (TNFα) [20]. Importantly, MPA inhibits the function of pDCs and reduces the production of IFNα in response to Toll-like receptor (TLR)-7, -8 and -9 ligands. Furthermore, MPA prevents the down-regulation of HIV-1 coreceptors CXCR4 and CCR5 on activated T cells and causes increased replication of HIV-1 in activated peripheral blood mononuclear cells (PBMCs) [20]. Immunosuppressive properties of DMPA have been consistently demonstrated in various models [4,5,16–18,20–29].

The effect of hormonal contraception on HIV-1 acquisition and transmission represents a critical global public health issue. Recent World Health Organization (WHO) meeting on programmatic and research priorities for contraception for women at risk of HIV identified the research addressing the association between various methods of hormonal contraception and HIV acquisition and transmission as a top priority, with an emphasis on injectables and other long-term methods [30]. Accumulated studies indicating the immunosuppressive properties of DMPA [4,5,16–18,20–29], and the epidemiological evidence demonstrating an association between DMPA use and increased risk of HIV-1 and other infections [5–13,31–35] strongly suggest that the use of DMPA should be discontinued, especially in areas with high HIV-1 prevalence. However, withdrawal of DMPA from family planning programs without offering equally effective forms of contraception is not warranted as it could result in a sharp increase in unwanted births, unsafe abortions, and maternal and infant mortality. In some regions up to nine additional maternal deaths could occur for every case of HIV-1 averted [36–38]. Replacement of DMPA with condoms would result in a significant increase of unintended pregnancies due to high failure rates [37]. Thus, it is critical to identify a contraceptive regimen that could effectively replace DMPA without exerting undesired side effects.

Most family planning programs strongly favor long-term methods of contraception due to higher efficacy, reliability, and ease of use. Norethisterone (NET)-based injectables are commonly used in resource-limited countries [1,3,9,31,39–41]. Levonogestrel (LNG) or etonogestrel (ETG)-releasing devices or implants are highly effective and reversible methods of long-term contraception [42–44]. ETG, LNG, and NET are considered for use in HIV-1-endemic areas; however, their safety in regard to the effect on immune system and HIV-1 transmission has not been validated [1,3,4,9,39,40,45]. Identification of contraceptives that do not suppress the protective properties of the immune system is critical for the selection of safe hormonal contraception in areas with high HIV-1 prevalence [4,36,37,40]. In order to find safe alternatives to DMPA, we analyzed the effect of commonly used progestins on the adaptive and innate immune system. Importantly, the presented data indicate that, at physiological levels, NET and LNG do not suppress cytokine production by activated T cells or pDCs.

2. Materials and methods

2.1. Study participants and sample collection

All procedures involving the use of human subjects were approved by the institutional review board of the University of Alabama at Birmingham. Informed consent was obtained from all participants. All volunteers were recruited at UAB, Birmingham, AL, USA. The volunteers were healthy premenopausal women; blood was collected at 10–22 days post last menstruation. One hundred milliliters of acid citrate dextrose-treated blood was collected by personnel trained in phlebotomy.

2.2. Materials

All cell culture reagents were obtained from Mediatech (Manassas, VA, USA), unless indicated otherwise. Etonogestrel was obtained from Bosche Scientific (New Brunswick, NJ, USA); all other synthetic hormones were purchased from Sigma-Aldrich (St. Louis, MO, USA). Antibodies for flow cytometry were purchased from eBioscience, (San Diego, CA, USA), unless listed otherwise. Progesterone (P4; pregn-4-ene-3,20-dione), medroxyprogesterone acetate (MPA; 17α-hydroxy-6α-methylprogesterone acetate), norethisterone (NET; 19-nor-17α-ethynyltestosterone),