FEMALE SEX HORMONES INFLUENCE VAGINAL HIV-1 INFECTION AND DISSEMINATION IN A HUMANIZED MOUSE MODEL


Kristen Mueller
McMaster University
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Presenter Disclosure

- I have no conflicts of interest to declare
Women & HIV

- Approximately 40% of HIV transmission occurs in the female genital tract
- 12.6 million cases of HIV are estimated to be a result of vaginal intercourse
- In 2015 women account for 25% of new HIV cases in Ontario
- OHTN has identified women as one of their 5 priority populations most affected by HIV
- Social & biological factors for increased infection rates

"This epidemic unfortunately remains an epidemic of women."

Michel Sidibé, Executive Director of UNAIDS
June 2010 United Nations News Centre
Biological Mechanism of HIV Infection

**Hormones**

- **Estrogen**: Protective effects against viral infection
  - Increase epithelial barrier integrity
  - Decrease levels of HIV target cells
  - Increase protease inhibitors

- **Progesterone**: Increased susceptibility to viral infections
  - Epithelial barrier remodeling/disruption
  - Immune cell recruitment
  - Increased HIV target cells

- **Hormonal Contraceptives**

Birse et al. *J. Virol* (Sep 2015)
Hormonal Contraception & HIV Risk

- Over 150 million women worldwide use hormonal contraceptive
- Approximately 50 million women use the injectable synthetic progestin Depot medroxyprogesterone acetate (DMPA)
- Heffron et al 2011: associated DMPA use with ~2-fold increase in risk of HIV-1 acquisition & transmission

PRESS STATEMENT
UNAIDS CALLS FOR URGENT ANALYSIS AND MORE RESEARCH ON HORMONAL CONTRACEPTIVES AND HIV INFECTION RISK
Hormonal Contraception & HIV Risk Research

Depot Medroxyprogesterone Acetate Increases Immune Cell Numbers and Activation Markers in Human Vaginal Mucosal Tissues

Neelima Chandra, Andrea Ries Thurman, Sharon Anderson, Tina Duong Cunningham, Nazita Yousefi, Christine Mauck, and Gustavo F. Doncel

Changes in Vaginal Microbiota and Immune Mediators in HIV-1-Seronegative Kenyan Women Initiating Depot Medroxyprogesterone Acetate

Alison C. Raxby, MD, MSc,* David N. Fredricks, MD,*†† Katherine Oden-Davis, PhD,§§ Kristjana Ásbjörnsdóttir, PhD,¶ Linnet Masese, PhD,¶ Tina L. Fiedler, BS,¶ Stephen De Rosa, MD,‡‡ Walter Jasko, MBChB, MTM, PhD,** James N. Kiarie, MBChB, MMPhD, MPH,¶¶¶ Julie Overbaugh, PhD,§§ and R. Scott McClelland, MD, MPH*|||

Medroxyprogesterone Acetate Regulates HIV-1 Uptake and Transcytosis but Not Replication in Primary Genital Epithelial Cells, Resulting in Enhanced T-Cell Infection

Victor H. Ferreira, Sara Dizell, Aisha Nazi, Jessica K. Kafka, Kristen Mueller, Philip V. Nguyen, Michel J. Tremblay, Alan Cochrane, and Charu Kaushic

Progestin-Containing Contraceptives Alter Expression of Host Defense-Related Genes of the Endometrium and Cervix

Gabriel A. Goldfin, BS¹, Fatima Barragan, BS¹, Joseph Chen, PhD, MS¹, Margaret Takeda, BA¹, Juan C. Irwin, MD, PhD¹, Jean Perry, RN, MS, NP¹, Ruth M. Greenblatt, MD³, Karen K. Smith-McCune, MD, PhD¹, and Linda C. Giudice, MD, PhD, MSc¹

CCR5 Expression Levels in HIV-Uninfected Women Receiving Hormonal Contraception


Hormonal Contraception and HIV-1 Infection: Medroxyprogesterone Acetate Suppresses Innate and Adaptive Immune Mechanisms

Richard P. H. Huijbersg, E. Scott Helton, Katherine G. Michel, Steffanie Sabbaj, Holly E. Richter, Paul A. Goepfert, and Zdenek Hel
Hormonal Contraception & HIV Risk

B. Adjusted hazard ratios for DMPA vs. women not using hormonal contraception – primary model

Overall 1.4 Hazard Ratio

Early Events of HIV infection in Women

Research Aim:
To study early events of HIV replication and spread in our model of heterosexual transmission using a humanized mouse model.
Humanized Mouse model of HIV infection

**Humanization of Mice**

- **Irradiation**
  - Newborn pups exposed to sub-lethal radiation
  - Remove "mouse" immune system

- **Reconstitution**
  - Pups injected with $1 \times 10^6 - 2 \times 10^6$ CD34-enriched human hematopoietic stem cells
  - Give mice "human" immune system

12 Week old Hu-Mice
- Reconstitution level confirmed in circulating PBMCs

HIV-1
- Intravaginal Inoculation
  - $10^3-10^5$ TCID$_{50}$/mL of NL4.3-Bal-Env HIV-1

1-12 Weeks
- Monitor Mice, Blood collection, Vaginal washes

Endpoint:
- Blood collection, Harvest Tissues
- Flow Cytometry, Histology, PCR

**Animals:**
- Male 4-6 weeks old nude SCID mice
- Newborn pups exposed to sub-lethal radiation
- Adult mice

**Cells:**
- CD34-enriched human hematopoietic stem cells
HIV-1 viral burden declines in vaginal lavage and increases in plasma following intravaginal infection.

**Vaginal Wash**

**Plasma**
Dissemination of HIV-1 following intravaginal infection of humanized mice

HIV-1 RNA (copies/mL)

1 week post infection – $10^3$

5 weeks post infection – $10^3$
Intravaginal HIV-1 infection in humanized mice leads to a decrease in circulating human CD4 T cells over time.
Intravaginal model of HIV infection in Humanized Mice

Following intravaginal exposure HIV establishes as a local infection in the vaginal tract of humanized mice that disseminates systemically to other tissues within the body within 3-5 weeks.

Humanized mice provide a good model to understand heterosexual HIV transmission in women.
The effects of sex hormones on intravaginal HIV infection in a humanized mouse model

1) Examine if different stages of the female reproductive cycle had any effect on susceptibility of humanized-mice to HIV-1

2) Compare susceptibility of hu-mice following DMPA administration

12 Week old Hu-Mice
Reconstitution level confirmed in circulating PBMCs

Vaginal wash for Estrus Staging

Estrus (Estrogen High)

Diestrus (Progesterone High)

DMPA Injection

HIV-1
Intravaginal Inoculation
$10^3$-$10^5$ TCID50/mL of NL4.3-Bal-Env HIV-1

1-5 Weeks
Monitor Mice, Blood collection, Harvest Tissues

Endpoint: Blood collection, Harvest Tissues
Flow Cytometry, Histology, PCR
Hormonal environment in mice determines infection rate in intravaginal HIV exposure

- Estrus: 0%
- Diestrus: 68%
- DMPA: 85%
HIV-1 levels in plasma is comparable between Diestrus and DMPA treated mice

1 Week Post-Challenge: $P=0.1979$

3 Weeks Post-Challenge: $P=0.5826$

5 Weeks Post-Challenge: $P=0.3993$
Dissemination of HIV-1 following intravaginal infection of Diestrus and DMPA treated mice
Uninfected DMPA treated humanized mice have increased levels of Target cells in blood
Summary

**Estrogen High**
- Studies suggest protection against viral infections
- Our mice model doesn’t get infected

**Progesterone High**
- Studies suggest susceptible period to viral infections
- We see local infection & then systemic spread

**DMPA treated**
- 1.4 fold increase risk of HIV acquisition
- We see comparable HIV levels & viral spread as Progesterone High mice
- We see increased infection rates & target cells in blood
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Canadian HIV Vaccine Initiative
www.chvi-icvv.gc.ca
### Table 1 | Contribution of HIV invasion sites to global HIV infections*  

<table>
<thead>
<tr>
<th>HIV invasion site</th>
<th>Anatomical sub-location</th>
<th>Type of epithelium</th>
<th>Transmission medium</th>
<th>Transmission probability per exposure event</th>
<th>Estimated contribution to HIV cases worldwide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female genital tract</td>
<td>Vagina</td>
<td>Squamous, non-keratinized</td>
<td>Semen</td>
<td>1 in 200–1 in 2,000</td>
<td>12.6 million</td>
</tr>
<tr>
<td></td>
<td>Ectocervix</td>
<td>Squamous, non-keratinized</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>Endocervix</td>
<td>Columnar, single layer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>Various</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male genital tract</td>
<td>Inner foreskin</td>
<td>Squamous, poorly keratinized</td>
<td>Cervicovaginal and rectal secretions and desquamations</td>
<td>1 in 700–1 in 3,000</td>
<td>10.2 million†</td>
</tr>
<tr>
<td></td>
<td>Penile urethra</td>
<td>Columnar, stratified</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>Various</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestinal tract</td>
<td>Rectum</td>
<td>Columnar, single layer</td>
<td>Semen</td>
<td>1 in 20 – 1 in 300</td>
<td>3.9 million§</td>
</tr>
<tr>
<td></td>
<td>Upper GI tract</td>
<td>Various</td>
<td>Semen</td>
<td>1 in 2,500</td>
<td>1.5 million</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Maternal blood, genital secretions (intrapartum)</td>
<td>1 in 5 – 1 in 10</td>
<td>960,000§</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Breast milk</td>
<td>1 in 5 – 1 in 10</td>
<td>960,000§</td>
</tr>
<tr>
<td>Placenta</td>
<td>Chorionic villi</td>
<td>Two layer epithelium (cytotrophoblast)</td>
<td>Maternal blood (intrauterine)</td>
<td>1 in 10 – 1 in 20</td>
<td>480,000§</td>
</tr>
<tr>
<td>Blood stream</td>
<td></td>
<td></td>
<td>Blood products, sharps</td>
<td>95 in 100 – 1 in 150</td>
<td>2.6 million§</td>
</tr>
</tbody>
</table>

*Table adapted from the UNAIDS/WHO AIDS epidemic update and REFS 121–128. †Includes men having sex with men (MSM), bisexual men and heterosexual men. §Includes MSM, bisexual men and women infected via anal receptive intercourse. ¶Mother-to-child transmission. ¶Mostly intravenous drug use, but includes infections by transfusions and health-care-related accidents. GI, gastrointestinal.

**Follicular Phase: Estrogen Dominant**

- Protease inhibitors
- Epithelial barrier integrity (cell-cell adhesion factors)
- Levels of cornification differentiation factors
- Amounts of proximal HIV target cells

**Luteal Phase: Progesterone Dominant**

- Protease mediated epithelial barrier remodeling/disruption
- Immune cell recruitment
- HIV target cells

WHO recommends women to discuss HIV risk and DMPA use with their doctor to determine if it is a suitable choice of contraceptive

DMPA users recommended to use barrier methods to prevent STI transmission

Insufficient evidence to globally condemn DMPA use

**Risks of an interaction between IHC and HIV risk** Butler et al.

*AIDS* 2013, 27:105–113
Expression of viral p24-antigen begins in the vaginal tract before spreading systemically.
HIV-1 infection is independent of the level of human CD45+ cells in the peripheral blood.
Dissemination of HIV-1 in NRG mice following intravaginal infection

**Based on 4th mouse 7/18 positive tissues....waiting for Toronto results – may change!**
HIV-1 levels in vaginal lavages lower in DMPA treated mice compared to Diestrus mice

1 Week Post-Challenge
P=0.3962

3 Weeks Post-Challenge
***
P=0.0005

5 Weeks Post-Challenge
*
P=0.0420
A. 10^5 Infectious Units

Vaginal lavage HIV-1 RNA (copies/mL)

B. 10^3 Infectious Units

Vaginal lavage HIV-1 RNA (copies/mL)

C. 10^5 Infectious Units

Plasma HIV-1 RNA (copies/mL)

D. 10^3 Infectious Units

Plasma HIV-1 RNA (copies/mL)