3D *IN VITRO* MODEL TO STUDY MILK-BORNE TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) - Abstract No. 812

S. Urdaneta Hartmann[1], M. Reginato[2], V. Holmes[1], P. Jain[3], B. Wigdahl[3] and M.K. Howett[1]

[1]College of Arts and Sciences, Department of Bioscience and Biotechnology, [2]College of Medicine, Department of Biochemistry, and College of Medicine, [3]Department of Microbiology and Immunology

MOTHER-TO-CHILD TRANSMISSION OF HIV-1 THROUGH BREASTFEEDING

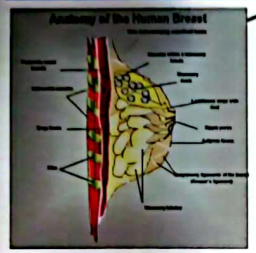
- In the absence of any intervention, 1/3 of infants born to HIV+ mothers become infected as well
- Half of these vertical transmission cases of HIV-1 are through breastfeeding
- The source of HIV-1 in milk and how breast milk becomes infected remains inconclusive
  - ➢Derived from virus circulating in the blood stream?
  - ➢The breast is a separate compartment for origin and replication of its own variant of HIV-1 (different from virus swarm in blood)?
- Cell-free virus and infected cells in breast milk can infect the infant
- Mammary epithelial cells are susceptible to HIV-1 infection and replication *in vitro*
- Prevention of transmission of HIV-1 through milk has not yet been successful (Hartmann et al., 2006a)
  - ➢There is not pharmacological therapy to prevent transmission through milk
  - ➢Heat treatment of milk inactivates HIV-1 but significantly destroys immunoglobulins in milk important for the infant's protection from infections
  - ➢We proposed treatment of milk with low doses (≤1%) of microbicides, like sodium dodecyl sulfate (SDS), which inactivates HIV-1 in milk within 10 min and conserves nutritional and immunoprotective functions of milk) (Urdaneta et al., 2005, and Hartmann et al., 2006b)

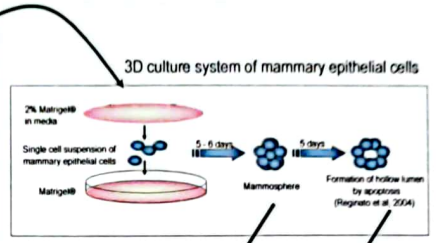
No *in vitro* or *in vivo* models exist to study the mother-to-child transmission of HIV-1 and its prevention.
Here we propose an in vitro model to study infection of the breast compartment by HIV-1.

OTHER PATHOGENS TRANSMITTED THROUGH BREASTFEEDING

HUMANS	ANIMALS
Human T cell leukemia virus type 1 (HTLV-1)	Mouse mammary tumor virus (MMTV)
Cytomegalovirus (CMV)	Caprine arthritis encephalitis virus (CAEV)
West Nile virus (one case reported)	Feline immunodeficiency virus (FIV)

MAMMOSPHERES A 3D CULTURE OF MAMMARY EPITHELIAL CELLS



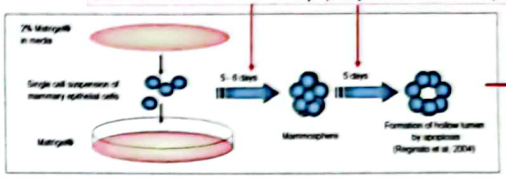


Applications of the *in vitro* 3D model
- Cell-cell signaling
- Breast cancer
- Mouse mammary tumor virus (MMTV)
- Mammary transmission of Caprine arthritis encephalitis virus (CAEV)

20x maginification of mammosphere stained with DAPI only (blue) or with DAPI and anti-laminin antibody (green)

PROPOSED USE OF MAMMOSPHERES TO STUDY HIV-1 INFECTION OF THE BREAST COMPARTMENT

Labeled virus or labeled infected lymphocytes at different time points



- Le Jan et al. (2005) have reported, using CAEV, that lymphocytes transmigrate through the matrix and can infect target cells.
- **Advantages:** Uses human breast cells, can incorporate infection by cell-free and/or cell-associated HIV-1, allows direct visualization of virus-cell interaction.
- Disadvantages of this system: Milk production has been achieved in mammospheres of other animal species (mice, goat), but not in human. Even though mammary epithelial cells are are susceptible to infection by HIV-1, they have not been specifically implicated in transmission of HIV-1. Other cellular components normally present in the mammary environment are missing.

ANTICIPATED RESULTS

We are interested in developing a model to study the mechanism of infection with HIV-1 of human breast compartment. We propose the use of this 3D *in vitro* model to study cell-viral interaction to determine the mechanism of infection of the breast compartment. If successful, this would open the window for understanding the cellular mechanism(s) involved in transmission of HIV-1 from mother to child through breastfeeding.

IN VIVO MODEL TO TEST THE USE OF SODIUM DODECYL SULFATE (SDS) AS A MICROBICIDE IN MILK FOR PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HUMAN IMMUNODEFICIENCY VIRUS (HIV-1) - Abstract No. 845

S. Urdaneta Hartmann[1], J.D. Trujillo[2], A. Argüello[3], and M.K. Howett[1]

[1]College of Arts and Sciences, Department of Bioscience and Biotechnology, [2]Utah State University, Utah Veterinary Diagnostic Laboratory, and [3]Universidad de Las Palmas de Gran Canaria, Department of Animal Science

SDS AS A MICROBICIDE AGAINST HIV-1 IN HUMAN MILK

We have previously reported on the microbicidal activity of SDS, a common ingredient in personal care products (e.g., toothpaste, shampoo, lotions) and is a protein denaturant and surfactant.

- ➢ ***Howett et al., 1999:*** SDS inactivates sexually transmitted pathogens (e.g.,HIV-1, HPV, HSV-2, *Chlamydia*) quickly and at low concentrations (≤1% SDS for 10min).
- ➢ ***Howett et al., 2002:*** At these treatment concentration, SDS is not toxic to the vaginal epithelium.
- ➢ ***Urdaneta et al., 2005 and Hartmann et al., 2006:*** SDS (≤1% within 10 min) also inactivates HIV-1 in human milk without significant decrease in milk's nutritional and protective functions. This treatment dose is within safe limits of ingestion of SDS in children, according the the United Nations Environmental Program (UNEP).

The use of microbicides to prevent mother-to-child transmission (MTCT) through breastfeeding is a novel concept that had not been explored to date. We propose that microbicidal treatment of HIV-1 infected breast milk with SDS may be a safe, effective and inexpensive way to prevent MTCT of HIV-1.
Here we propose a method for pre-clinical testing in vivo of SDS as a microbicide in milk.

THE PROPOSED MODEL: MILK-BORNE TRANSMISSION OF CAEV IN GOATS

- CAEV (Caprine Arthritis Encephalitis Virus), like HIV-1, is a lentivirus from the retrovirus family.
- Seroprevalent world wide (in US is 31-81%).
- CAEV is mainly transmitted by breastfeeding of newborn kids with infected colostrum.
- Only 1/3 of the goats develop observable disease (encephalitis in young kids and chronic debilitating arthritis in adult goats). Infected goats need to be sacrificed to prevent further transmission.
- The established method to prevent transmission in goats is to separate the kids from their mothers immediately after birth and bottle -feed them pasteurized goat milk.
- Pasteurization destroys CAEV, but it also destroys immunoglobulins important for the immune protection of the kids against infections.

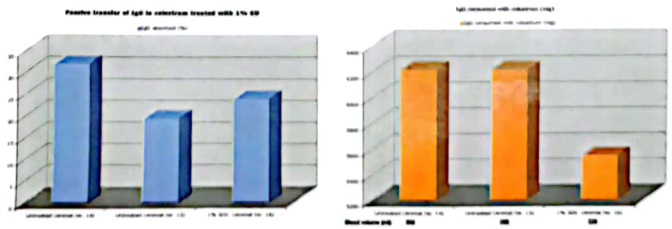
SPECIFIC AIMS OF THIS PRE-CLINICAL STUDY

1. Determine microbicidal inactivation of CAEV in vitro with SDS.
2. Determine acceptance of intake of SDS-treated colostrum by newborn kids, and the effect of SDS-treatment on nutritional and immunological components in goat colostrum.
3. Determine the efficacy of SDS to prevent transmission of CAEV in vivo.

PRELIMINARY RESULTS (SPECIFIC AIM 2)

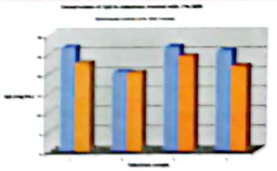
- Groups of 3-4 newborn kids (*Capra hircus*) were fed either untreated colostrum or 1% SDS-treated colosutrm.
- Blood samples were taken at 0, 24, 48, and 72 h for IgG quantification (radial immunodiffusion) and electrophoretic profile of immunoglobulins.
- IgG and total immunoglobulins were quantified in treated and untreated colostrum.

Passive transfer of IgG in 1% SDS-treated (10 min at 37°C) colostrum is within normal range

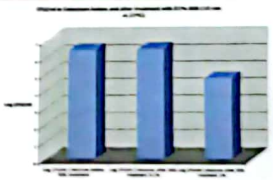


PRELIMINARY RESULTS (SPECIFIC AIM 2) - Continuation

90% of IgG in colostrum is conserved after treatment with 1% SDS (10 min at 37 C)



Significant decrease in bacteria in colostrum after treatment with 1% SDS (10 min at 37 C)



INITIAL CONCLUSIONS, PREDICTIONS AND FUTURE DIRECTIONS

- 1% SDS in colostrum was accepted by kids. No side effects were observed.
- 1% SDS may be the ideal treatment concentration.
- Treatment concentration needs to be confirmed with in vitro inactivation assays with CAEV. Experiments are on the way.
- If in vitro and in vivo results are positive, experiments to compare efficacy of SDS in vivo with that of pasteurization will be designed.

This an adequate in vivo method for determining efficacy of SDS in preventing MTCT of milk-borne infection. SDS may prove to be a better alternative than pasteurization for goat farmers. Further experiments are needed.

Department of Bioscience & Biotechnology