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Feasibility of radiolabeled small molecule permeability as a quantitative measure of microbicide candidate toxicity

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Abstract

Objective: To determine the feasibility of using quantitative changes in vaginal permeability to small molecules as a measure of candidate microbicide toxicity.
Study design: Controlled, open-labeled, prospective study. Seven healthy women received a single vaginal dose of hydroxyethylcellulose gel (HEC), nonoxynol-9 (N-9) or K-Y Jelly. Each gel was radiolabeled with a small molecule (^{99m}Tc-DTPA) followed by 12-h blood and urine collection. Pharmacokinetic (PK) parameters of ^{99m}Tc-DTPA were calculated to compare the impact of each gel on vaginal permeability. Each woman served as her own control. The Friedman test with *post hoc* Wilcoxon test was used to detect differences among the gels.
Results: Vaginal permeability of ^{99m}Tc-DTPA was highest for the N-9 radiolabel. N-9 plasma area under the concentration curve was 2.7-fold higher ($p=.04$), and peak concentration was threefold higher ($p=.04$) compared to HEC. There were no significant PK parameter differences between HEC and K-Y Jelly or between N-9 and K-Y Jelly. Cumulative dose-adjusted median (interquartile range) 12-h timed urine gamma activity was 66.70×10^{-4} μCi (27.90–152.00) following HEC dosing, 103.00×10^{-4} μCi (98.20–684.00) following N-9 gel dosing and 20.30×10^{-4} μCi (11.10–55.90) following K-Y gel dosing. The differences between urine HEC and K-Y Jelly ($p=.047$) and between N-9 and K-Y Jelly ($p=.016$) were statistically significant.
Conclusions: It is feasible to measure differences in vaginal permeability among vaginal gels using a radiolabeled small molecule, though there are permeability differences that require a nuanced understanding of gel composition to interpret the results.
Implications: Establishing the safety of both vehicle and active pharmaceutical ingredient is an essential task in microbicide development, to be determined as soon as possible. This study suggests that a combination of microbicide toxicity assessments, that is, cervicovaginal permeability, inspection and histopathology, may need to be studied simultaneously.

Keywords: HIV; N-9; Permeability; HIV; Microbicide; Spermicide

1. Introduction

Microbicides are topically applied drugs that prevent HIV sexual transmission through inhibition of HIV penetration into or replication within target cells and tissues. CAPRISA 004, a randomized, placebo-controlled clinical study of vaginal 1% tenofovir gel dosed vaginally both before and after sex, demonstrated a 39% reduction in HIV transmission in heterosexual women, which provided clinical proof-of-concept for topical microbicides in the prevention of HIV infection [1]. In two other randomized controlled trials, vaginal 1% tenofovir gel did not protect women in modified intent-to-treat analyses, but reduction in HIV risk was demonstrated in *post hoc* analyses among women who had detectable drug levels indicative of higher levels of adherence [2,3].

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Microbicide development requires early identification of gel vehicles or active pharmaceutical ingredients (API) that might be toxic to the epithelium. Similarly, interpretation of microbicide trial results requires considering confounding sources of toxicity, including the gel vehicle and *para*-sexual activities (i.e., sexual lubricants, douching) to avoid incorrect attribution of toxicity to the microbicide's API. An example is nonoxynol-9 (N-9), which was used for decades as an over-the-counter spermicidal agent. In a study among commercial sex workers, there was a 50% increased risk of HIV acquisition among N-9 recipients [4].

In vitro experiments suggest that for some molecules, the vaginal mucosa is more permeable than colonic or intestinal tissue [5] and is relatively comparable to the buccal mucosa [6]. These colon-versus-vaginal relationships are drug and vehicle specific. Our group has used permeability as a metric to compare changes in colonic mucosal integrity after dosing rectal microbicide candidates [7]. The purpose of this study was to compare vaginal permeability of a radiolabeled small molecule added to three gels — hydroxyethylcellulose (HEC), N-9 and K-Y Jelly™ — as a simple, noninvasive assessment of the potential impact of each gel on cervicovaginal permeability.

2. Materials and methods

This was a comparative, open-label prospective study comparing vaginal permeability among three radiolabeled gels. Healthy women age 18 to 45 years with regular menstrual cycles were invited to participate. Women agreed to use effective contraception and abstain from vaginal intercourse, vaginal products or vaginal sex toy insertion for 7 days prior to and following dosing visits. Women with a sexually transmitted infection (STI), vaginal candidiasis or bacterial vaginosis within 8 weeks of enrollment were excluded. Known history of genital herpes, current urinary tract infection, cervicovaginal procedure within 3 months, hysterectomy, pregnancy, breastfeeding, undiagnosed irregular menses, urogenital malformations and allergy to gel components were additional exclusion criteria. Written informed consent was obtained. The study was approved by the Johns Hopkins Medicine Institutional Review Board.

2.1. Study gels

The three vaginal gels were HEC, N-9 (Gynol II Ortho-McNeil-Janssen Pharmaceuticals, Inc., Titusville, NJ, USA) and K-Y Jelly™. HEC gel, which is an *iso*-osmolar (290–300 mOsm/kg) nondetergent gel similar to universal placebo, was selected as the negative control. Because HEC is *iso*-osmolar and does not contain any ingredients at concentrations that are expected to have possible effects on the mucosa, we did not expect that HEC would be associated with any permeability changes. Two percent N-9, which is a hyper-osmolar (1200 mOsm/kg) detergent gel, was selected as the positive control because it demonstrated detectable permeability changes in our prior rectal permeability study [7]. K-Y Jelly, which is a hyper-osmolar (2007 mOsm/kg) gel, was selected for comparison with the positive and negative controls to understand if the hyperosmolar character of K-Y Jelly by itself, but without the detergent properties of N-9, would result in permeability changes [8]. A 3.5 mL of each gel was radiolabeled with technetium-99 m diethylene triamine pentaacetic acid (^{99m}Tc-DTPA) that has a molecular weight of 487.31 and was supplied by a commercial radiopharmacy (Cardinal Health, Beltsville, MD, USA) [9]. DTPA is highly water soluble and is cleared 100% by glomerular filtration [10,11]. The dose planned for delivery to the research participant was 500 μCi ^{99m}Tc. The actual dose delivered and retained is described below.

2.2. Procedures

A single 3.5-mL dose of a gel was administered at separate visits and administered in the same sequence — HEC gel, N-9 and K-Y Jelly. Dosing visits were scheduled at the same time in each participant's menstrual cycle to avoid any potential impact of hormonal variation on vaginal permeability. The menstrual phase was not standardized for the entire sample as some participants underwent procedures only in the follicular phase while others underwent procedures only in the luteal phase. At least a 3-week washout period was scheduled between doses, which was more than sufficient for complete clearance of all products from the prior visit, complete radioactive decay of the administered isotope and complete healing of vaginal mucosa. A negative serum pregnancy test was required before gel application, and a Foley catheter was inserted to obtain an accurate measurement of urine ^{99m}Tc, while avoiding urine contamination by vaginal gel leakage. Radiolabeled gel was injected into the posterior vaginal fornix using a luer-lock applicator (product 35–1107; Professional Compounding Centers of America, Houston, TX, USA) attached to the dosing syringe. The residual radioactivity in each syringe was measured using a dose calibrator (CRC 15-W; Capintec, Ramsey, NJ, USA) and the time of measurement recorded. The retained dose was calculated from the dose administered minus the residual radioactivity in the syringe after dosing.

Participants remained supine for the first hour after dosing to facilitate specimen collection and allowed to ambulate *ad lib* afterwards. Sanitary napkins were used by all participants and were collected and measured for radioactivity to account for leakage of the isotope-labeled gel from the vagina. Whole venous blood was collected predose and at 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 6, 8, 10 and 12 h after gel insertion; plasma was separated for measurement of gamma activity. Urine was collected predose and 2, 4, 8 and 12 h after gel insertion. After 12 h, residual gel was rinsed from the vagina using 10 mL of an isotonic salt balanced solution (Normosol-R™, Hospira, Inc.).