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Hyperosmolal vaginal lubricants markedly reduce epithelial barrier properties in a three-dimensional vaginal epithelium model

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ABSTRACT

Most of the widely used vaginal lubricants in the U.S. and Europe are strongly hyperosmolal, formulated with high concentrations of glycerol, propylene glycol, polyquaternary compounds or other ingredients that make these lubricants 4 to 30 times the osmolality of healthy vaginal fluid. Hyperosmolal formulations have been shown to cause marked toxicity to human colorectal epithelia in vivo, and significantly increase vaginal transmission of genital herpes infections in the mouse/HSV model. They also cause toxicity to explants of vaginal epithelia, to cultured vaginal epithelial cells, and increase susceptibility to HIV in target cells in cell cultures. Here, we report that the osmolality of healthy vaginal fluid is 370 ± 40 mOsm/Kg in women with Nugent scores 0–3, and that a well-characterized three-dimensional human vaginal epithelium tissue model demonstrated that vaginal lubricants with osmolality greater than 4 times that of vaginal fluid (> 1500 mOsm/Kg) markedly reduce epithelial barrier properties and showed damage in tissue structure. Four out of four such lubricants caused disruption in the parabasal and basal layers of cells as observed by histological analysis and reduced barrier integrity as measured by trans-epithelial electrical resistance (TEER). No epithelial damage to these layers was observed for hypo- and iso-osmolal lubricants with osmolality of < 400 mOsm/Kg. The results confirm extensive reports of safety concerns of hyperosmolal lubricants and suggest the usefulness of reconstructed in vitro vaginal tissue models for assessing safety of lubricants in the absence of direct clinical tests in humans.

1. Introduction

Hyperosmolal lubricants containing spermicides such as nonoxynol-9 (N-9) induce exfoliation or shedding of the outer layers of the human colorectal epithelium and reduce its barrier properties [1–3]; both these toxic effects are thought likely to increase risk of acquiring and transmitting sexually transmitting infections such as HIV and Herpes Simplex Virus (HSV). Mucosal irritation has been shown to increase with increasing hyperosmolality of several commercially available vaginal lubricants using the slug mucosal irritation assay [4] and perhaps most importantly, to significantly increase vaginal transmission of genital herpes infections in the mouse/HSV model [5]. Lastly, use of some vaginal lubricants is associated with increased incidence of bacterial vaginosis (BV) [6–10]. BV is strongly associated with increased risk of HIV-1 [11] as well as gonorrhea, trichomonas is, upper-tract infections that contribute to preterm deliveries and perinatal complications, pelvic inflammatory disease, and other gynecological and urinary tract infections (see [12]). Here we used a three-dimensional reconstructed model of human vaginal epithelium (EpiVaginal, MatTek Corporation, Ashland, MA), a well-characterized in vitro tissue model for detecting the irritation potential or toxic effects of vaginally applied lubricants. Tests such as these are needed in the continuing absence of clinical safety tests in humans.

The usual toxicity test recommended by the FDA for vaginally formulations/lubricants is the rabbit vaginal irritation model. Unlike in humans, the distal rabbit vagina also acts as a urethra [13] and is directly exposed to the marked variations in the osmolality of urine. In contrast, the human vagina is not normally exposed to hyperosmolal fluids. Moreover, the pH of the rabbit vagina is essentially neutral (pH 7.0), whereas the healthy human vagina is distinctly acidic, with a pH ranging from 3.2 to 4.2 with ~1% lactic acid [12]. The human vaginal epithelium is unique in that it provides a high concentration of glycogen that can support mono-microbial cultures of lactobacilli that acidify the vagina with lactic acid to levels that likely provide significant first-line of protection against many pathogens, including HIV, HSV, chlamydia, and gonorrhea [12] as well as protection against the polymicrobial communities of BV bacteria [14,15]. Neither the rabbit vagina, nor most cell monocultures are exposed to this level of lactic

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acid acidity, and therefore may not reliably reflect how the human vagina will respond to vaginal lubricants formulated to match, and support, human vaginal acidity, nor is the rabbit vagina likely to be as susceptible to hyperosmolal lubricants as the human vagina. Thus, three-dimensional vaginal tissue models derived from human vaginal and cervico-vaginal cells represent a useful tool for detecting toxic effects of vaginal lubricants in the continuing absence of human clinical toxicity trials.

The osmolality of at least 44 personal lubricants have been reported [5,16–18]. Thirty-eight of these (86%) are hyperosmolal, and only six are approximately iso-osmolal or hypo-osmolal. The hyperosmolal lubricants range from 4 to more than 30 times the osmolality of vaginal fluids (370 ± 40 mOsm/kg as reported here). Since most of the over-the-counter (OTC) lubricants exceed the osmolality of vaginal fluid, the World Health Organization (WHO) recommended on an interim basis a maximum acceptable osmolality limit of 1200 mOsm/kg [17,19]. In addition 45 of 52 lubricants are formulated with a pH > 4 well above the protective lactic acid acidity of the healthy human vagina, pH < 4 with ~1% lactic acid [12].

Even though the toxic effects of hyperosmolal lubricants have been examined following rectal application in humans, the toxic effects of commercially available lubricants have not been tested clinically in humans, nor on 3D vaginal tissue models. The overall aim of this investigation was to use one of the best characterized in vitro vaginal tissue models to detect disruption of epithelial barrier functions caused by widely available lubricants following a single topical exposure as a function of their osmolality.

2. Material and methods

2.1. Osmolality of vaginal fluid in humans

Vaginal fluid collection was performed as published previously [20]. Briefly, undiluted vaginal fluid samples, averaging 0.5 g per sample, were obtained from women of reproductive age by using a self-sampling menstrual collection device following protocols approved by the Institutional Review Board of the Johns Hopkins University (protocol # HIRB00000526). Donors inserted the device into the vagina for about 30 s, removed it, and placed it into a 50 ml centrifuge tube. Samples were centrifuged at 200*g* for 2 min to collect the vaginal fluid. Samples were collected at random times throughout the menstrual cycle; the menstrual cycle date was estimated based on the last menses date reported by the donor and normalized to a 28-day cycle. Samples that were non-uniform in color or consistency were discarded. Donors stated they had not used vaginal products nor participated in unprotected intercourse within 3 days prior to donating. A total of 8 donors were recruited at the Johns Hopkins University Homewood campus, a population with very low incidence of BV and all 8 donors had healthy vaginal microbiota (Nugent scores 0–3). Each donor provided an average of 5 samples. Osmolality was measured using a VAPRO® 5520 vapor pressure osmometer (ELITech Group, Logan, UT) at room temperature and calibrated at 100, 290, and 1000 mOsm/Kg. For vaginal secretions, a Wiretrol (Drummand, Broomall, PA) was used to deposit 10 µl on the test disc of paper. For watery test agents, 10 µl was delivered with the manufacturer's pipet. For gels too thick for this delivery method, the test disc of paper was submerged in the gel, and squeezed between two layers of Parafilm "M"® (Bemis, Neenah, WI) to leave ~10 µl of gel saturated in the disc paper (thereby avoiding contaminating the thermocouple with the gel). For hyperosmolal products, the product was diluted on a wt/wt basis with deionized water to bring the osmolality into the ~300 mOsm/Kg range, and then corrected for this dilution factor.

2.2. Source of vaginal epithelial cells

Human vaginal-ectocervical (VEC) tissue was obtained from otherwise healthy women (age 29–48) undergoing hysterectomies for benign indications via the National Disease Research Interchange (NDRI, Philadelphia, PA) following Internal Review Board (IRB) approval. The ectocervix was used as a source of vaginal epithelial cells; the ectocervix forms a part of the posterior vaginal wall and is covered by stratified squamous epithelium that is histologically indistinguishable from the epithelium lining the lateral vaginal wall. It is also the vaginal site most exposed to pooled vaginal lubricants, and is likely to be susceptible to toxic effects of vaginally administered products. Epithelial cell isolation and expansion was performed using optimized cells expansion medium (MatTek Corporation) as described previously [21].

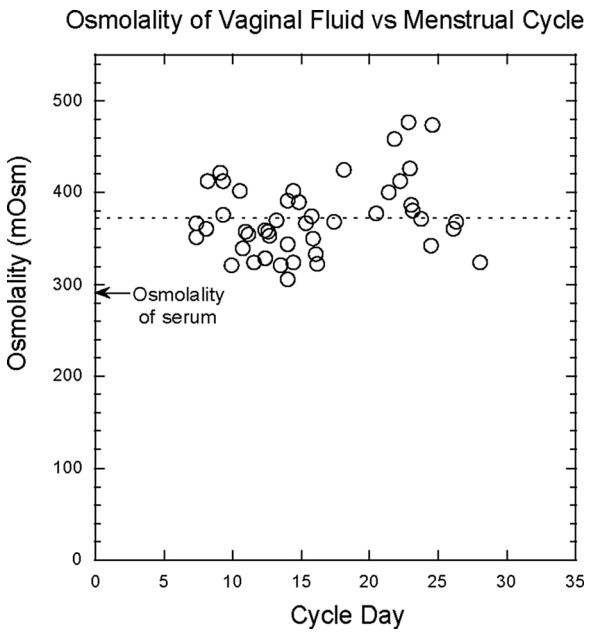

Fig. 1. Osmolality of unmodified vaginal secretions collected at random times during the menstrual cycles of women.

2.3. In vitro tissue reconstruction

Cryopreserved cells from a single donor were thawed and plated on 150-mm petri dishes. When the cell density reached 60–70% confluence, cells were trypsinized, counted and seeded at a density of 5×10^5 cells/cm^2 onto polycarbonate tissue culture treated microporous membrane cell culture inserts (MatTek, Ashland, MA). Inserts were cultured at 37 °C, 5% CO_2, 98% rH for 4 days submerged and 7 days at the air liquid interface using a serum free VEC-100-MM (MatTek Corporation, Ashland, MA) differentiation medium to produce the VEC tissue. This culture procedure yields a well-stratified and non-cornified vaginal-ectocervical tissue model (VEC, EpiVaginal) that recapitulates the phenotypic and structural features of *in vivo* vaginal epithelium.

2.4. Test products and dose volume

In this study we chose several widely available over-the-counter (OTC) vaginal lubricants characteristic of two groups, those with ~iso-osmolal formulations, and those formulated with hyperosmolal concentrations of glycerol or propylene glycol: The ~iso-osmolal formulations tested were Good Clean Love (GCL, Eugene, OR), Aloe Cadabra (Seven Oaks Farm, Ventura, CA), Pre-Seed (Lil' Drug Store Products, Inc, Cedar Rapids, IA), and Restore (GCL, Eugene, OR). The hyperosmolal lubricants tested were RepHresh (Lil' Drug Store Products, Inc, Cedar Rapids), K-Y Jelly (Reckitt Benckiser LLC, Parsippany, NJ), ID Glide (Westridge Laboratories, Inc., Newport