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In vitro inhibition of human papillomavirus following use of a carrageenan-containing vaginal gel☆,☆☆,☆☆☆

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HIGHLIGHTS

- We evaluated the ability of a carrageenan containing sexual lubricant to inhibit PsV16.
- Carrageenan levels decreased over time, PsV16 inhibition remained high eight hours after insertion of the carrageenan gel.
- This is the first clinical study directly looking at carrageenan potential for HPV prevention.

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ABSTRACT

Objective. To assess in vitro efficacy of Divine 9, a carrageenan-based vaginal lubricant that is being studied as a microbicide to inhibit HPV16 pseudovirus (PsV) infection.

Methods. Sexually active US women between 19 and 35 years without prior HPV vaccination or cervical intraepithelial neoplasia were instructed to use Divine 9 vaginally with an applicator either before sex only or before and after intercourse. Women who applied a single dose of gel returned for cervicovaginal lavage (CVL) collection 1, 4 or 8–12 h after intercourse versus those who applied gel before and after intercourse returned 1, 4 or 8–12 h after the second gel dose. Carrageenan concentrations were assessed using an ELISA assay and the inhibitory activity was assessed using a PsV-based neutralization assay against HPV16 infection. Carrageenan concentrations and the percentage of PsV16 inhibition were compared using the Wilcoxon rank sum test.

Results. Thirteen women were enrolled and thirty specimens from different time-points were assessed. 87% of CVL samples had detectable carrageenans with levels decreasing over time from intercourse. 93% of CVL samples had detectable PsV16 inhibition with median inhibition of 97.5%. PsV16 inhibition decreased over time, but remained high, with median inhibition of 98.1%, 97.4% and 83.4% at 1, 4 and 8–12 h, respectively. Higher carrageenan concentrations were associated with higher levels of PsV16 inhibition (rho = 0.69).

Conclusions. This is the first report of a human study investigating in vitro HPV inhibition of a carrageenan-based vaginal lubricant with CVL collected after sexual intercourse. We demonstrate excellent efficacy in preventing PsV16 infection.

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1. Introduction

Human papillomavirus (HPV) is the most common sexually transmitted infection in the United States and is necessary for the development of cervical cancer. Although HPV vaccination is highly effective, HPV and cervical disease remain a major public health concern in the US, in part, due to the poor uptake of the HPV vaccine. The Centers for Disease Control and Prevention (CDC), for example, reported an uptake rate of only 40% among US adolescents between 13 and 17 years of age as of 2015 [1]. Vaccination of women older than 26 years of age is not currently recommended, due to diminished efficacy in older age groups. Thus, many young women in the US remain unvaccinated and at least several generations of women will not fully benefit from HPV vaccine. Furthermore, the quadrivalent and bivalent vaccines used through early 2015 in the US targeted only a limited number of HPV types associated with cervical disease, and whether revaccination with nonavalent (9-valent) (or other future vaccines) will be recommended is unknown. While HPV vaccination rates are higher in certain other developed nations, similar limitations regarding the use of quadrivalent or bivalent HPV vaccines and the large number of unvaccinated women 27 years of age or older, still apply.

However, the greatest public health concern regarding HPV infection and cervical disease is in areas or countries of medium and limited resource settings, given limited access to HPV vaccine, compounded by limited access to cervical cancer screening and treatment options. In many settings, but in particular in limited resource settings, condom use is a primary means of preventing HPV and cervical disease by decreasing exposure to infectious particles.

Consistent condom use appears to be moderately successful in preventing HPV transmission, with a 70% decrease in transmission reported in young women whose partners used a condom 100% of the time [2]. Thus, development and testing of condom-compatible microbicides to prevent HPV infection has significant clinical implications.

Carrageenans (CG) are a family of sulfated polysaccharides that are extracted from edible red seaweed. They are commonly used in the food industry as thickening agents as well as formulated as personal lubricants. Carraguard, a CG based vaginal gel, has been shown to be safe and well tolerated [3–5]. Although a large randomized, placebo-controlled trial of Carraguard failed to show Carraguard efficacy in preventing HIV infection in women [6], in vitro and in vivo preclinical evaluation has suggested that CG may have high efficacy in blocking HPV infection [7–13]. Furthermore, in a subgroup analysis of the HIV prevention trial, there was a 60% reduction in the prevalence of high-risk HPV infection in women who consistently used Carraguard [14]. However, no a priori trials have been performed to investigate the efficacy of CG to decrease HPV transmission.

The current study was designed to assess genital tract concentrations of carrageenan as well as inhibition of HPV infection using an in vitro pseudovirion assay after application of a commercially available carrageenan-containing vaginal lubricant prior to penetrative sexual intercourse.

2. Materials and methods

2.1. Eligibility criteria

After obtaining approval from the Albert Einstein College of Medicine Institutional Review Board, women were recruited between February and May 2014 from gynecology clinics at Montefiore Medical Center in Bronx, NY. Eligible subjects were healthy, sexually active women between the ages of 19–35, with an intact cervix, engaging in at least three intercourse events per month, and using an effective contraceptive method for at least 3 months. Exclusions included a history of surgical excision or hysterectomy for cervical intraepithelial neoplasia, a history of HPV vaccination, malignancy, HIV or other immunosuppressive disease, genital warts or ulcers, pelvic inflammatory disease, pregnancy, and breastfeeding. Informed consent was obtained from each participant prior to enrollment. At the screening visit, all subjects underwent laboratory testing including: urine pregnancy test, oral (saliva-based) HIV screening test, liquid-based monolayer Pap testing with HPV typing using a brush, testing for gonorrhea and chlamydia and cervicovaginal lavage (CVL) with 10 cm^3 saline. Screening for Trichomonas and/or a wet mount for bacterial vaginosis and candidiasis was performed for symptomatic patients.

2.2. Intervention gel

Divine 9 containing Carragel (CarraShield Labs, Orlando, FL) is a non-contraceptive water-based lubricant that is clear, odorless and tasteless and made with a mixture of lambda and kappa carrageenans naturally derived from red seaweed. The gel is formulated with 2% carrageenans, which is similar to gels that have previously been investigated in microbicide trials [6,15,16]. It is manufactured in a U.S. FDA GMP-compliant facility and approved as a Class II medical device. All of its ingredients are GRAS (Generally Recognized as Safe) under CFR Title 21 and are food-grade. It has been directly marketed to consumers for over ten years and there have been no reported safety concerns to date.

This interventional gel was supplied to participants in single-use disposable applicators from HTI Plastics (Lincoln, NE) containing 2.5 ml of gel, which delivers on average 2 ml of gel. Divine 1, a Carrageenan-Free Gel (CarraShield Labs, Orlando, FL), a gel with similar rheological properties without carrageenan, was used as a control for the in vitro studies.

2.3. Intervention

Participants were assigned alternately to one of two groups: The *precoital* group was instructed to insert Divine 9 within 12 h prior to vaginal intercourse, have intercourse, and return at an assigned time-point. A second group was instructed to insert one dose of Divine 9 within 12 h before vaginal intercourse, have intercourse, and then insert a second dose of gel as soon as possible, but within 12 h after sex and no more than two doses in a 24-hour period. Hence, the dosing strategy is referred to as *BAT24*. As per participant report, most inserted gel immediately before and in the *BAT24* group, immediately after sex. After an act of sexual intercourse the participants called the research team and those in the *precoital* group were asked to return to the clinic for CVL collection using 10 ml of 0.9% saline solution at 1, 4 or 8–12 h after intercourse, while those in the *BAT24* group returned for CVL collection 1, 4 or 8–12 h after insertion of the second dose of Divine 9. CVLs were stored at −80 °C.

For each coital act, only one time-point was measured as gel is washed out with CVL. However, participants were allowed to return for repeat measurements at different time-points after a coital act as long as there was at least a one-week washout period between study visits. Patients were compensated at IRB-established rates for each return visit.

2.4. In vitro assay for anti-HPV activity

HPV16 pseudovirions (PsV) were prepared based on reagents and methods obtained from the Schiller lab, as previously described [17]. Briefly, 293TT cells were co-transfected with codon-modified HPV16 capsid genes (L1 and L2) together with a reporter plasmid encoding secreted human placental alkaline phosphatase (SEAP). Efficient purification of the HPV16 PsV was achieved by Optiprep™ (Sigma-Aldrich, Allentown, PA) density gradient ultracentrifugation (iodixanol). A titration of the HPV16 PsV stock was routinely tested to determine the minimum amount of HPV16 PsV required giving a robust inhibition signal at the neutralization assay.

Serial dilutions of Divine 9, Divine 1, and PC-525, another CG-containing gel developed by Population Council, New York, NY, were evaluated. After dilution testing of HPV16 PsV preparations to identify

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