Carrageenan Is a Potent Inhibitor of Papillomavirus Infection

Christopher B. Buck[1], Cynthia D. Thompson[1], Jeffrey N. Roberts[1], Martin Müller[2], Douglas R. Lowy[1], John T. Schiller[1*]

1 Laboratory of Cellular Oncology, National Cancer Institute, Bethesda, Maryland, United States of America, **2** Forschungsschwerpunkt für Angewandte Tumorvirologie, Deutsches Krebsforschungszentrum, Heidelberg, Germany

Certain sexually transmitted human papillomavirus (HPV) types are causally associated with the development of cervical cancer. Our recent development of high-titer HPV pseudoviruses has made it possible to perform high-throughput in vitro screens to identify HPV infection inhibitors. Comparison of a variety of compounds revealed that carrageenan, a type of sulfated polysaccharide extracted from red algae, is an extremely potent infection inhibitor for a broad range of sexually transmitted HPVs. Although carrageenan can inhibit herpes simplex viruses and some strains of HIV in vitro, genital HPVs are about a thousand-fold more susceptible, with 50% inhibitory doses in the low ng/ml range. Carrageenan acts primarily by preventing the binding of HPV virions to cells. This finding is consistent with the fact that carrageenan resembles heparan sulfate, an HPV cell-attachment factor. However, carrageenan is three orders of magnitude more potent than heparin, a form of cell-free heparan sulfate that has been regarded as a highly effective model HPV inhibitor. Carrageenan can also block HPV infection through a second, postattachment heparan sulfate–independent effect. Carrageenan is in widespread commercial use as a thickener in a variety of cosmetic and food products, ranging from sexual lubricants to infant feeding formulas. Some of these products block HPV infectivity in vitro, even when diluted a million-fold. Clinical trials are needed to determine whether carrageenan-based products are effective as topical microbicides against genital HPVs.

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Introduction

Papillomaviruses are a diverse group of nonenveloped DNA viruses that infect the skin and mucosal tissues of a range of vertebrate species, including humans. A group of genital mucosotropic human papillomavirus (HPV) types are etiologic agents responsible for virtually all cases of cancer of the uterine cervix, as well as a substantial fraction of other ano-genital and head-and-neck cancers (reviewed in [1]). Cancer-associated genital HPV types, as well as another subset of HPV types associated with the development of benign genital warts (condyloma accuminata), are generally transmitted through sexual contact. Infection with genital HPV types is very common, with an estimated lifetime risk of infection of about 75% [2]. Although most genital HPV infections are subclinical and self-limiting, a subset of persistently infected individuals have lesions that progress to premalignancy or cancer.

Recent meta-analyses have suggested that condoms are, at best, only marginally effective for preventing the sexual transmission of HPV [3,4]. However, a highly effective group of prophylactic HPV vaccines are expected to become publicly available in the near future [5]. Two possible drawbacks to these vaccines are that they are expected to be relatively expensive (at least initially) and are likely to be papillomavirus type-restricted in their protection. Thus, the vaccines may not initially be available to women in all parts of the world and may not offer protection against all cancer-associated HPV types. Inexpensive condom-compatible compounds that could function as broad-spectrum topical microbicides targeting sexually transmitted HPVs might therefore serve as useful adjuncts to vaccination programs.

In vitro analysis of papillomavirus infection has historically been hampered by the fact that key events in the late phase of the viral lifecycle, such as the expression of the capsid proteins L1 and L2, require cellular differentiation in the upper layers of the stratified squamous epithelial tissues that the viruses inhabit (reviewed in [6]). As a consequence, papillomaviruses cannot replicate in conventional monolayer cell cultures. Investigation of the assembly and entry phases of the papillomavirus lifecycle has recently been simplified by the development of high-yield methods for producing papillomavirus-based gene transfer vectors, known as pseudoviruses (PsV), using conventional monolayer cell lines [7,8]. We have used PsV to develop a high-throughput screening method to identify and compare compounds with the potential to block papillomavirus infectivity in vitro [9].

Previous studies have shown that sulfated polysaccharides, such as heparin, cellulose sulfate, and dextran sulfate, can block the infectivity of papillomaviruses [10–12]. For many classes of virus, including papillomaviruses, initial attachment of the virion to cultured cell lines is thought to be mediated

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Abbreviations: BPV1, bovine papillomavirus type 1; CHO, Chinese hamster ovary; GAG, glycosaminoglycan; GFP, green fluorescent protein; HPV, human papillomavirus; HSPG, heparan sulfate proteoglycan; IC_{50}, 50% inhibitory concentration; JORRP, juvenile onset recurrent respiratory papillomatosis; PsV, pseudovirus

* To whom correspondence should be addressed. E-mail: schillej@dc37a.nci.nih.gov

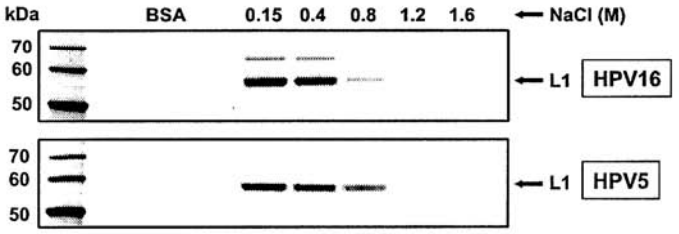

Figure 3. Capsids Bind Carrageenan

Carrageenan beads were incubated with HPV16 or HPV5 capsids in buffers with the NaCl concentration shown. The beads were washed, then bound capsids were eluted and visualized in stained SDS-PAGE gels. Bovine serum albumin (BSA, 65 kDa) was used as a control for nonspecific binding to the beads. For HPV16, an L2 band can be seen above the L1 band.

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products were extremely potent inhibitors, with IC_{50} values occurring at dilutions of a few million-fold (Table 3). Two highly inhibitory European brands, Bioglide and Bioglide Anal, list carrageenan as an ingredient. The other components in these two products, water, glycerol, and xanthan gum, were noninhibitory when tested individually (Tables 2 and 3).

One US product, Divine N° 8, uses the term "natural kelp extract" in its ingredient list. Although it is not clear what type of algal polysaccharide this term refers to, the high potency of Divine N° 8 in the HPV16 PsV inhibition assay strongly suggests that the product contains carrageenan. An unscented version of the product, Divine N° 9, does not list ingredients on its packaging or at its manufacturer's website, but its high potency suggests that it, too, may contain carrageenan. The inhibition curves for the Bioglide and Divine lubricants were similar to what would be expected if the products were composed of roughly 1%–3% carrageenan, a typical concentration range used to achieve gelation.

The packaging of a third US product, ForPlay Gel Plus, lists carrageenan as its fifth ingredient, behind several nonsulfated thickening agents. ForPlay Gel Plus did not display detectable inhibitory effects, even at dilutions as little as a hundred-fold. It is possible that ForPlay Gel Plus either contains very little carrageenan, or contains a less-inhibitory type of carrageenan. Alternatively, interactions between carrageenan and other compounds in ForPlay Gel Plus may abrogate the inhibitory effects of the carrageenan.

Lubricant brands containing other algal polysaccharides, such as agar and algin, were less effective for blocking the HPV16 PsV in the inhibition assay, consistent with the observation that these compounds are less inhibitory than carrageenan when tested individually (Table 2).

Several lubricant gels that do not contain sulfated polysaccharides were ineffective for blocking the HPV16 PsV at tested doses. Ortho Options Conceptrol, a contraceptive gel containing the detergent spermicide nonoxynol-9, does not contain sulfated polysaccharides. The fact that Conceptrol was ineffective for blocking the HPV16 PsV at noncytotoxic doses (Table 3) is consistent with a previous report demonstrating that nonoxynol-9 is not effective for blocking papillomavirus infectivity [28].

Another common use for carrageenan is as a stabilizing agent in milk-based products, including infant feeding formulas. The use of infant formulas containing carrageenan might thus be a factor in vertical transmission of HPVs, since such transmission could involve establishment of initial infection in infants' oral mucosa. We therefore tested several brands of infant formula using the HPV16 PsV inhibition assay. Formulas containing carrageenan displayed significant inhibitory effects, while fresh milk and infant formulas without carrageenan did not display detectable inhibitory effects at tested doses (Table 3).

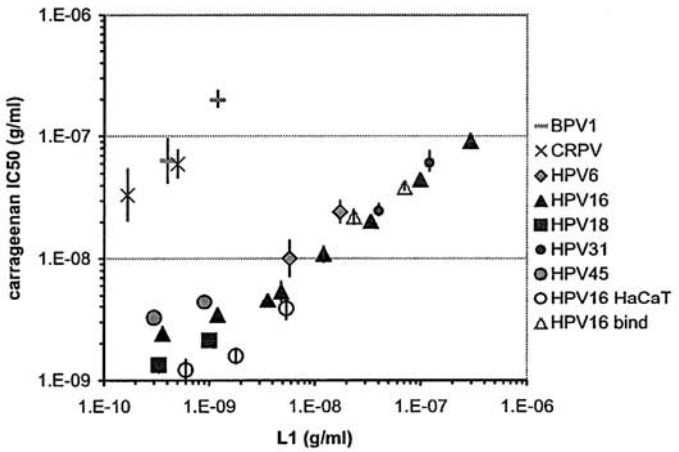

Figure 4. Standardized Carrageenan IC_{50} for Various Papillomavirus Types

Points represent carrageenan IC_{50} of infectivity, except for empty red triangles, which represent the carrageenan IC_{50} of cell binding for HPV16 capsids covalently linked to a fluorescent dye. Empty circles represent carrageenan IC_{50} of infectivity observed using HaCaT cells instead of HeLa cells. Error bars represent the 95% CI for the IC_{50}.

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Discussion

In this report we demonstrate that carrageenan, an inexpensive commercial thickening agent extracted from seaweed, is an exceptionally potent inhibitor of papillomavirus infectivity in vitro. Carrageenan was found to be active against a range of common sexually transmitted HPV types that can cause cervical cancer and genital warts. Since carrageenan is generally recognized as safe for food and topical applications, it is an appealing candidate for use as a broad-spectrum topical microbicide to block HPV transmission.

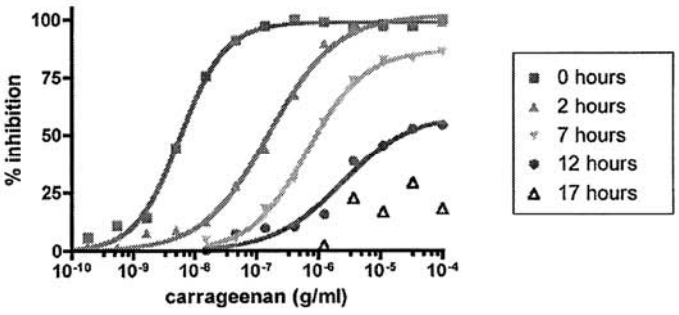

Figure 5. Carrageenan Addition Time Course

Cells were incubated with HPV16 PsV for 2 h, followed by washout of the virus inoculum. Carrageenan was added at the timepoints shown, where time zero represents initial PsV inoculation.

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Table 3. Inhibitory Effects of Consumer Products

Group	Product	Manufacturer	Compound of Interest	IC_{50}	95% CI
Lubricant	Aquaglide	Bioglide	—	—	—
	Bioglide	Bioglide	Carrageenan	6×10^6	$4.6–6.7 \times 10^6$
	Bioglide Anal	Bioglide	Carrageenan	7×10^6	$6.1–7.7 \times 10^5$
	Divine N° 8	DivineTimes	Natural kelp extract	8×10^6	$6.8–8.7 \times 10^6$
	Divine N° 9	DivineTimes	Unknown	1×10^7	$9.1–11 \times 10^6$
	ForPlay Gel Plus	Trimensa	Carrageenan	—	—
	Glycerol (glycerin)	SigmaAldrich	—	—	—
	Intimate Infusions Almost Naked	SacredMomentsProducts	Agar	2,400	1,800–3,100
	K-Y Jelly	JNJ	—	—	—
	Ortho Options Conceptrol	Ortho-McNeilPharmaceutical	Nonoxynol-9	—	—
	Stimula	Stimula-US	Algin	560	360–870
	Surgilube	Fougera	—	—	—
Infant formula	Carnation Nonfat Dry Milk	Nestle	—	<100	—
	Enfamil (concentrate)	MeadJohnson	Carrageenan	3×10^4	$2.5–2.9 \times 10^4$
	Enfamil (dry)	MeadJohnson	—	<100	—
	Fat-free milk	Safeway	Lactoferrin	<100	—
	Good Start Supreme (concentrate)	Nestle	—	<100	—
	Similac Advance (ready)	Abbott	Carrageenan	3×10^4	$3.1–3.6 \times 10^4$

Values are expressed as fold dilution.
None of the products scored as cytotoxic at dilutions ≥100-fold, except Conceptrol, which was cytotoxic at dilutions ≤900-fold and Surgilube, which was cytotoxic at the 100-fold dilution.
The results do not imply endorsement or nonendorsement of any product.
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Some, but not all, carrageenan-containing over-the-counter sexual lubricant gels we tested were extremely effective for blocking the infectivity of an HPV16 reporter pseudovirus in vitro. These results raise the possibility that use of such lubricant products, or condoms lubricated with carrageenan-based gels, could block the sexual transmission of HPV. However, in the absence of clinical efficacy data, it would be inappropriate to recommend currently available products for use as topical microbicides.

Carrageenan is also active in vitro and in murine model systems against other viruses, including herpes simplex viruses and some strains of HIV-1 [29–34]. However, in vitro IC_{50} values for carrageenan inhibition of herpes simplex virus and HIV-1 infectivity are about a thousand-fold higher than the IC_{50}s we have observed for carrageenan inhibition of genital HPVs in vitro.

It is important to emphasize that cell culture systems may not fully represent some aspects of HPV infection of keratinocytes in vivo. However, our group has recently developed a pseudovirus-based murine genital challenge model for initial HPV infection (unpublished data). This animal model system should be useful for investigating of the potential efficacy of carrageenan for blocking HPV transmission in vivo.

A clinical trial focused on the effectiveness of a κ/λ-carrageenan preparation as a topical microbicide is currently in progress in South Africa. A recent patent application by the trial's organizers (http://www.popcouncil.org) contains a claim of carrageenan as a papillomavirus inhibitor, but the potency of the inhibitory effect was not indicated [35]. Since the principal focus of the ongoing trial is the efficacy of carrageenan against HIV-1, it may be necessary to develop additional clinical trials specifically focused on the in vivo efficacy of well-defined carrageenan preparations against HPVs. The high rate of acquisition of genital HPV infection in young adult populations (reviewed in [2]) might make it possible to perform short-duration clinical efficacy trials with relatively small numbers of human subjects.

Our results show that the principal mechanism by which carrageenan blocks papillomavirus infectivity is via the direct binding of carrageenan to the viral capsid. The binding of carrageenan appears to block interactions between the capsid and cell-surface HSPG attachment factors. Although the presence of HSPGs on the cell surface significantly enhances papillomavirus binding to and infection of most types of cultured cell lines [10,11,36,37], in this report we have used GAG-negative cells to demonstrate conclusively that HSPG attachment factors are not strictly required for infection to occur. A similar situation has been described for certain strains of HIV-1, particularly lab-adapted HIV-1 strains, for which HSPGs are thought to serve as attachment factors that facilitate (but are ultimately dispensable for) the in vitro infection of some cultured cell lines [38,39].

In addition to blocking the initial interaction between papillomavirus virions and HSPGs, carrageenan also exerts a second HSPG-independent inhibitory effect. This secondary inhibitory effect could be due to occlusion of virion surfaces involved in binding to cellular proteins involved in the infectious process. Alternatively, carrageenan might interfere with the development of needed conformational changes within the virion. Since it is possible that HPVs use alternative, non-HSPG attachment factors in vivo [36,37], the existence of a postattachment, GAG-independent inhibitory effect increases the likelihood that carrageenan might ultimately be effective as a topical microbicide against HPVs.

Although carrageenan was highly effective for neutralizing five different genital HPV types in vitro, it was substantially less potent against several papillomavirus types tropic for nongenital skin. Since common genital HPVs occupy a single genus, and the three nongenital papillomavirus types we have