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Effect of undecylenic acid as a topical microbicide against genital herpes infection in mice and guinea pigs

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Abstract

There is increasing interest in the use of topical microbicides to help prevent the spread of sexually transmitted diseases (STD). Undecylenic acid (UA), a monosaturated fatty acid, is the active ingredient in a number of over-the-counter (OTC) antifungal spray powders, that also exhibits in vitro antibacterial and antiviral activity, including herpes simplex virus (HSV) activity. We, therefore, evaluated UA as a topical microbicide against genital HSV infection using the murine and guinea pig models of genital herpes. Mice were administered a 20% solution of UA in polyethylene glycol (PEG) vehicle, vehicle alone or phosphate buffered saline (PBS) intravaginally immediately prior to vaginal challenge with HSV-2. Pre-treatment with UA decreased the number of mice that became infected ($P < 0.001$ vs. PBS or vehicle control), developed symptoms ($P < 0.001$) or died ($P < 0.001$). However, when treatment was extended to either 5 min prior to or after viral inoculation, protection was lost. Similar findings were found using the guinea pig model, where UA treatment completely prevented HSV-2 vaginal infection when given immediately prior to HSV-2 inoculation ($P < 0.001$ vs. PBS or vehicle control). Thus, UA, an approved OTC medication, provided significant protection against HSV disease and infection only when applied immediately before viral inoculation, indicating that better formulations were needed to extend the duration of protection.

Keywords: Herpes simplex virus; Microbicides; Genital herpes; Mice; Guinea pigs

1. Introduction

The incidence of sexually transmitted disease (STD) continues to increase. Thus, there is a growing interest in the development of topical microbicides, chemoprophylactic agents that can be used locally to decrease the risk of acquiring an STD. Microbicides are appealing because they could be used as protection by females without male consent, thus overcoming some of the problems associated with condom use (Stein, 1990;

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Elias and Coggins, 1996; Farr et al., 1996).The World Health Organization, the US Department of Health and Human Resources, as well as the National Institute of Allergy and Infectious Diseases have identified the development of female-controlled topical microbicides as a high priority (Lange et al., 1993; International Working Group on Vaginal Microbicides, 1996). A successful topical microbicide should prevent the spread of multiple STD pathogens, but be safe and well tolerated even following frequent applications (Rosenthal et al., 1998).

One strategy for developing topical microbicides has been to identify agents like detergent or surface active agents that disrupt the outer envelope or membrane of the organism. One such candidate, Nonoxynol-9, the active ingredient in many commercially available spermicides (Weir et al., 1994), has been reported to provide protection against several STDs in animal models and human trials (Rosenberg et al., 1992; Moench et al., 1993; Whaley et al., 1993; Weir et al., 1994; Zeitlin et al., 1997). Frequent application of Nonoxynol-9, however, resulted in local vaginal irritation which could increase the risk of acquiring an STD, particularly HIV (Kreiss et al., 1991; Niruthisard et al., 1991; Roddy et al., 1993). A variety of other microbicides have been tested in vitro (Pauwels and De Clercq, 1996; Zeitlin et al., 1997), but few have been evaluated in vivo.

The safety record of a variety of over-the-counter (OTC) topical antifungal agents containing undecylenic acid (UA), another surface active agent and its antiviral activity suggested it might be a potentially safe and effective microbicide. UA is a monosaturated fatty acid with antifungal, antibacterial and antiviral activity (Shafran et al., 1997). It has been shown to have in vitro activity against herpes simplex type 1 (HSV-1) and HSV-2 (Huffman et al., 1989), as well as in vivo activity against HSV-1 (Sidwell et al., 1989). In a recent clinical evaluation, UA significantly reduced the incidence and duration of HSV shedding, but did not effect healing or progression of lesions in patients with recurrent herpes labialis (Shafran et al., 1997). When treatment was initiated during the prodrome, however, some therapeutic effects were noted. Thus, it appeared that early therapy was effective, suggesting that prophylaxis should be evaluated. In the experiments reported here, we used the mouse and guinea pig models of genital herpes to determine the potential effectiveness of UA as a topical microbicide.

2. Materials and methods

2.1. Viruses and cells

HSV-2 strain 186 (Milligan and Bernstein, 1995) and HSV-2 strain MS (American type culture collection, Rockville, MD) used in animal studies were prepared by culture in low-passage rabbit kidney (RK) cells. Stock virus was maintained frozen (− 80°C). Primary RK cells were prepared as previously described (Stanberry et al., 1987) and maintained in Eagle's basal medium supplemented with 10% fetal bovine serum.

2.2. Undecylenic acid

UA was obtained from Sigma (St. Louis, MO) and prepared as a 20% (W/V) solution in propylene glycol (Ruger Chemical, Irvington, NJ). Animals were treated by intravaginal instillation of the material at varying times before or after intravaginal inoculation with HSV-2. Mice received 0.015 ml and guinea pigs 0.1 ml of UA or placebo.

2.3. Mouse model of genital HSV-2 infection

Female Swiss Webster mice weighing 18–21 g (Harlan, Indianapolis, IN) were administered 0.1 ml of a suspension containing 3 mg medroxyprogesterone acetate (Upjohn Pharmacia, Kalamazoo, MI) by subcutaneous injection in the shoulder region 7 days and 1 day prior to virus challenge to increase susceptibility to vaginal HSV infection (Milligan and Bernstein, 1997). Following intraperitoneal injection of 0.25 ml of a solution containing 6.5 mg/ml sodium pentobarbital, the vagina was pre-swabbed with a moistened calcium alginate tipped swab and then with a dry swab. Animals were inoculated by instillation of 0.015 ml of a suspension containing 4.0 $\log_{10}$ PFU HSV-2 strain 186 after treatment (Milligan and Bernstein, 1995).

Vaginal swab samples were collected from all animals on day 2 post-inoculation and stored frozen (− 80°C) until assayed for the presence of virus by culture on susceptible RK cell monolayers (Milligan and Bernstein, 1997). The mice were evaluated to day 21 PI for evidence of symptomatic infection and mortality.

2.4. Guinea pig model of genital HSV-2 infection

Hartley guinea pigs, weighing 275–350 g (Charles River Breeding Laboratory, Wilmington, MA), were treated with 0.1 ml of UA or placebo instilled intravaginally following rupture of the vaginal closure membrane with a moistened calcium alginate tipped swab into the vaginal vault. The animals were then immediately inoculated, by intravaginal instillation, with 0.1 ml of a suspension containing 6.0 $\log_{10}$ pfu HSV-2 strain MS as previously described (Stanberry et al., 1987). Vaginal swab samples were collected from all animals on days 1 and 2 PI and stored frozen (− 80°C) until assayed for the presence of virus by culture on RK cell monolayers (Stanberry et al., 1987).

The guinea pigs were examined daily and the severity of primary genital skin disease was quantified using a lesion score scale, ranging from 0 for no disease to 4 representing severe vesiculoulcerative disease of the perineum (Stanberry et al., 1987). After recovery from primary disease, animals were observed daily from days 15–28 PI for evidence of spontaneous recurrent herpetic lesions (Stanberry et al., 1987).

2.5. Statistics

Incidence data were compared by Fisher's exact test. Comparisons of multiple groups were made by one-way ANOVA with Bonferroni correction. All comparisons were two-tailed.

3. Results

3.1. Efficacy in the mouse vaginal challenge model

In two initial studies, mice were intravaginally administered 15 μl UA solution, the propylene glycol vehicle alone or PBS, immediately (within 30 s) prior to HSV-2 inoculation. The results within treatment groups were comparable between the two experiments, so the results have been combined. Animals which survived to the end of the study and were asymptomatic, were defined as infected if virus was isolated from vaginal swab obtained 2 days after virus inoculation. By this definition, 27 of 29 PBS treated animals became infected (Table 1). Treatment with the propylene glycol vehicle significantly ($P < 0.05$) reduced the incidence of infection (21 of 31), compared to animals treated with PBS; however, there was no significant difference in survival between the two groups. In contrast, both the incidence of infection (3 of 31) and survival (3 of 31) in UA treated animals was significantly ($P < 0.001$) reduced compared to either PBS or vehicle controls.

We next examined whether the time of UA application could be extended before or after virus challenge in the mouse model. Consistent with the results from the first experiment, treatment with UA immediately prior to inoculation provided almost complete protection against infection and complete protection against death and disease. When UA was administered 5 min prior to virus challenge, however, it failed to protect against infection or to increase survival, although the mean day of death was significantly extended compared to controls (Table 2). When UA was administered 5 min after virus challenge, there was no change in the infection or survival rate or the mean day of death compared to controls (Table 2).

Table 1
Effect of undecylenic acid on genital HSV-2 infection in mice

Group[a]	*N*	Infected[b]	Symptoms	Died
Vehicle	31	21*	19	18
UA-0	31	3**	3**	3**
PBS	29	27	22	21

[a] Treatment was administered immediately prior to intravaginal HSV-2 inoculation.
[b] Animals which survived and were asymptomatic were defined as infected if virus was isolated from day 2 vaginal swabs
* $P < 0.05$ compared to PBS; ** $P < 0.001$ compared to vehicle or PBS.

Table 2
Effect of time of treatment with undecylenic acid on genital HSV-2 infection in mice

Group	*N*	Infected[a]	Symptoms	Died	MDD[b]
(A) Pre-therapy					
Vehicle	16	16	11	11	11.5 ± 0.5
UA–immediately pre-inoculation	16	0*	0*	0*	—
UA–5 min pre-inoculation	16	15	15	12	17.6 ± 0.7*
PBS	15	15	15	15	11.8 ± 0.7
(B) Post-therapy					
Vehicle	16	16	16	12	12.0 ± 1.0
UA–immediately pre-inoculation	16	2*	0	0*	—
UA–5 min after inoculation	16	14	12	12	15.5 ± 1.3
PBS	15	14	14	14	13.2 ± 1.1

[a] Animals which survived and were asymptomatic were defined as infected if virus was isolated from day 2 vaginal swabs.
[b] Mean day of death ($\pm$ S.E.).
* $P < 0.001$ vs. vehicle or PBS.

3.2. Efficacy in the guinea pig model of genital herpes

Guinea pigs were treated immediately (within 30 s) before HSV-2 inoculation and followed for primary and recurrent genital disease. As seen in Table 3, treatment with UA completely prevented infection (defined as recovery of HSV from vaginal swabs obtained on the first 2 days following viral inoculation) and acute or recurrent disease. The vehicle provided no protection from infection or acute or recurrent HSV disease.

4. Discussion

There is great interest in developing safe, effective, female-controlled methods that will prevent the acquisition of STDs. The limited use of condoms and the high prevalence of non-consensual sex, strongly suggest the need for an STD prophylactic method controlled by women. Further, although condoms can reduce the risk of pregnancy and STDs, there are personal, cultural and social barriers that interfere with a woman's ability to negotiate the use of condoms.

An acceptable vaginal microbicide must be safe even when used once or even multiple times per day (reviewed in Rosenthal et al., 1998). These agents must not damage the vaginal mucosa because inflammation, erosions or ulcers might increase the risk of acquisition of HIV or other STDs (Niruthisard et al., 1991; Roddy et al., 1993). Further, they should maintain other natural protective barriers such as the normal flora and acidic pH of the vagina. The ideal microbicide should be colorless, odorless, tasteless, inexpensive, stable and easy to store. It should be able to produce its effects immediately but remain active for a prolonged period. Products that are active pre- and post-coitus would be advantageous (Rosenthal et al., 1998). Spermicidal activity is not a prerequisite and, in fact, the lack of spermicidal activity might be preferred, especially if the product could be given either alone or formulated with a spermicide.

Currently, there are no safe and effective vaginal microbicides available. The product evaluated here, UA, has many of the properties of a successful microbicide outlined above. It was protective against HSV genital infections (defined as recovery of virus from vaginal swabs) in both mice, reducing infection from 95 to 8% (92% efficacy) and guinea pigs, reducing infection from 100 to 0%, when given immediately prior to viral inoculation. It is a clear,

Table 3
Effect of undecylenic acid on primary genital HSV-2 infection in guinea pigs

Group[a]	N	Infected[b]	Primary genital skin disease		Recurrent genital skin disease
			Incidence	Severity[c]	
Vehicle	12	10	7	7.1 ± 2.0	7[d]
UA-0	12	0*	0*		0**
PBS	12	12	11	10.3 ± 0.9	10

[a] Treatment was administered immediately prior to intravaginal HSV-2 inoculation.
[b] Animals that were asymptomatic were defined as infected if virus was isolated from day 1 or 2 vaginal swabs.
[c] Mean $\pm$ S.E., severity measured as the area under the lesion score–day curve.
[d] One animal developed a secondary bacterial infection of the perineum and could not be evaluated for recurrences. One animal without primary disease developed a recurrence.
* $P < 0.001$ vs. vehicle or PBS.
** $P < 0.005$ vs. vehicle or PBS.

odorless compound with an acidic pH. It is sold as an OTC remedy for fungal infections of the skin and therefore has a long history of safety although application to a mucosal surface is not well characterized and further extensive evaluation of safety prior to use as a vaginal microbicide would be needed. Initial indications reveal that this might indeed be a problem, as minor irritation was noted in a report of treatment for orolabial HSV infections (Shafran et al., 1997). Our animal evaluations did not reveal obvious effects on the vaginal mucosa, but therapy was only given once and no microscopic exam was performed.

The major concern raised by our initial evaluation is the duration of protection provided by UA. Thus, although complete protection was seen when it was given just prior to viral inoculation, protection was not seen when treatment preceded viral challenge by just 5 min. These results must be interpreted with caution as the formulations used have not been optimized and indeed were given as a 20% solution in propylene glycol. Further research is needed to determine if optimal formulations for this agent can prolong it's activity as well as that of other compounds that have also shown a limited duration of activity (Reising et al., 1998, unreported observation). Thus, it is possible that the lack of prolonged activity was due to rapid clearance of the compound from the vagina or from inactivation. The use of gels, foams or films should be evaluated to determine which provides optimal protection and safety. The use of timed release formulations should also be considered. The failure of treatment given 5 min post viral inoculation most likely reflects a lack of activity on virus that has already attached to cells. Similar observations of decreased activity were noted with Nonoxynol-9, another agent that disrupts the outer surface of viruses, when it was used 15 min after viral inoculation compared to use immediately before viral challenge (Whaley et al., 1993).

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References

Elias, C.J., Coggins, C., 1996. Female-controlled methods to prevent sexual transmission of HIV. AIDS 10 (3), S43–S51.

Farr, G., Castro, L.A.A., Disantostefano, R., Claassen, E., Olguin, F., 1996. Use of spermicide and impact of prophylactic condom use among sex workers in Santa Fe de Bogota, Columbia. Sex. Transm. Dis. 23, 206–212.

Huffman, J.H., Sidwell, R.W., Huffman, M.A., Radov, L.A., Watkins, B.E., Kinsolving, C.R., 1989. Virucidal and antiviral activity of undecylenic acid (UDA) vs. enveloped and nonenveloped DNA and RNA viruses (abstract), Proceedings of the International Symposium on Antiviral Chemotherapy, Porto Cervo, Italy.

International Working Group on Vaginal Microbicides, 1996. Recommendations for the development of vaginal microbicides. AIDS 10, 1–6.

Kreiss, J., Ngugi, E., Holmes, K., Guinan, M.E., Stone, K.M., Peterson, H.B., 1991. Efficacy of nonoxynol-9 contraceptive sponge use in preventing heterosexual acquisition of HIV in Nairobi prostitutes. J. Am. Med. Assoc. 268, 477–482.

Lange, J.M.A., Karam, M., Piot, P., 1993. Boost for vaginal microbicides against HIV. Lancet 341, 1356.

Milligan, G.N., Bernstein, D.I., 1995. Generation of humoral responses against herpes simplex virus type 2 (HSV-2) in the murine female genital tract. Virology 206, 234–241.

Milligan, G.N., Bernstein, D.I., 1997. Interferon gamma (IFNγ) enhances resolution of herpes simplex virus type 2 (HSV-2) infection of the murine genital tract. Virology 229, 259–268.

Moench, T.R., Whaley, K.J., Mandress, T.D., Bishop, B.D., Witt, C.J., Cone, R.A., 1993. The cat/feline immunodeficiency virus model for transmucosal transmission of AIDS: nonoxynol-9 contraceptive jelly blocks transmission by an infected cell inoculum. AIDS 7, 797–802.

Niruthisard, S., Roddy, R.E., Chutivongse, S., 1991. The effects of frequent nonoxynol-9 use on the vaginal and cervical mucosa. Sex. Transm. Dis. 18, 176–179.

Pauwels, R., De Clercq, E., 1996. Development of vaginal microbicides for the prevention of heterosexual transmission of HIV. J. Acq. Immun. Defic. Syndr. Human Retrovirol. 11, 211–221.

Reising, S.F., Bourne, K.Z., El-Awar, F.Y., Bourne, N., Stanberry, L.R., 1998, Evaluation of Triclosan as a possible intravaginal microbicide: in vitro and in vivo activity against herpes simplex virus type 2 (HSV-2) (Abstract No. 139), Eleventh International Conference on Antiviral Research, San Diego, CA.

Roddy, R.E., Cordero, M., Fortney, J.A., 1993. A dosing study of nonoxynol-9 and genital irritation. Int. J. STD AIDS 4, 165–170.

Rosenberg, M.J., Davidson, A.J., Chen, J., Judson, F.N., Douglas, J.M., 1992. Barrier contraceptives and sexually transmitted diseases in women: a comparison of female-dependent methods and condoms. Am. J. Public Health 82, 669–674.

Rosenthal, S.L., Cohen, S.S., Stanberry, L.R., 1998. Topical microbicides: current status and research considerations for adolescent girls. Sex. Transm. Dis. 25, 368–377.

Shafran, S.D., Sacks, S.L., Aoki, F.Y., Tyrrell, D.L., Schlech, W.F. III, Mendelson, J., Rosenthal, D., Gill, M.J., Bader, R.L., Chang, I., 1997. Topical Undecylenic acid for herpes simplex labialis: A multicenter, placebo-controlled trial. J. Infect. Dis. 176, 78–83.

Sidwell, R.W., Huffman, J.H., Radov, L.A., Watkins, B.E., Kinsolving, C.R., 1989, Inhibition of type 1 herpesvirus-induced lesions on guinea pigs by topically applied undecylenic acid (abstract), Proceedings of the International Symposium on Antiviral Chemotherapy, Porto Cervo, Italy.

Stanberry, L.R., Bernstein, D.I., Burke, R.L., Pachl, C., Myers, M.G., 1987. Recombinant herpes simplex virus glycoprotein vaccine protects against initial and recurrent genital herpes. J. Infect. Dis. 155, 914–920.

Stein, Z.A., 1990. HIV prevention: the need for methods women can use. Am. J. Public Health 80, 460–462.

Weir, S.S., Feldblum, P.J., Zekeng, L., Roddy, R.E., 1994. The use of nonoxynol-9 for protection against cervical gonorrhea. Am. J. Public Health 84, 910–914.

Whaley, K.J., Barratt, R.A., Zeitlin, L., Hoen, T.E., Cone, R.A., 1993. Nonoxynol-9 protects mice against vaginal transmission of genital herpes infections. J. Infect. Dis. 168, 1009–1011.

Zeitlin, L., Whaley, K.J., Hegarty, T.A., Moench, T.R., Cone, R.A., 1997. Tests of vagina microbicides in the mouse genital herpes model. Contraception 56, 329–335.