Effect of a Cellulose Acetate Phthalate Topical Cream on Vaginal Transmission of Simian Immunodeficiency Virus in Rhesus Monkeys

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Antimicrobial Agents and Chemotherapy, Nov. 2000, p. 3199–3202

Received 1 December 1999/Returned for modification 9 March 2000/Accepted 28 July 2000

Human immunodeficiency virus type 1 (HIV-1) infection continues to spread in developing countries, mostly through heterosexual transmission. The development of a safe and cost-effective topical microbicide, effective against a range of STDs including HIV-1, would greatly impact the ongoing epidemic. When formulated in a vehicle, a micronized form of cellulose acetate phthalate (CAP), which is an inactive pharmaceutical excipient, has been shown to inactivate HIV-1, herpes simplex virus types 1 and 2, cytomegalovirus, Neisseria gonorrhoeae, Trichomonas vaginalis, Haemophilus ducreyi, and Chlamydia trachomatis in vitro. Formulated CAP was also shown to be effective against herpes simplex virus type 2 in vivo. Here we show that a formulation of CAP protected four of six rhesus monkeys from vaginal infection with simian immunodeficiency virus. Thus, CAP may be a candidate for use as a topical microbicide for preventing HIV-1 infection in humans.

In developing countries, heterosexual transmission is responsible for the majority of new human immunodeficiency virus type 1 (HIV-1) infections. In addition, sexually transmitted diseases (STDs) have also been shown to facilitate HIV-1 infection (24, 25, 30). The over-the-counter contraceptive nonoxynol-9 (N9), which inactivates viral and bacterial STDs in vitro (6, 7, 10, 29) and is effective against simian immunodeficiency virus (SIV) in vivo (13, 14), has been widely evaluated clinically as a candidate topical microbicide (27, 28, 30). N9, however, can cause irritation of the vaginal mucosa and can alter the vaginal flora, potentially increasing the transmission of HIV-1 and other STDs (28, 29). Therefore, the evaluation of additional prophylactic agents with broad-spectrum anti-STD activity is warranted. An ideal candidate microbicide should be safe for repeated use, should not alter the vaginal mucosa or flora, and should be cost-effective to produce.

We previously reported that a modified protein from whey and milk, 3-hydroxyphthaloyl-β-lactoglobulin (designated 3HP-β-LG), suspended in phosphate-buffered saline and administered prior to and after intravaginal inoculation with SIV, was effective in preventing SIV transmission in 50% of the female rhesus monkeys tested (34). While 3HP-β-LG has demonstrated broad-spectrum antiviral activity (8, 9, 20–22), it has not been effective against bacterial STDs (A. R. Neurath, unpublished data). We have therefore continued to explore inexpensive agents that are produced from widely available resources with activity against a wide range of STDs.

Cellulose acetate phthalate (CAP) is an inactive pharmaceutical excipient commonly used in the production of enteric tablets and capsules. When formulated in a vehicle, a micronized form of CAP has been shown to inactivate HIV-1, herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), cytomegalovirus, Neisseria gonorrhoeae, Trichomonas vaginalis, Haemophilus ducreyi, and Chlamydia trachomatis in vitro (23). Formulated CAP has also recently been shown to be effective against HSV-2 in vivo (4). Another advantage of CAP is that it does not appear to affect lactobacilli, part of the natural vaginal flora which contributes to the resistance to STDs (23).

In this study, we used the SIV female rhesus monkey model of heterosexual HIV transmission to evaluate the efficacy of CAP in a glycerol-based cream containing povidone plus crospovidone (CAP:I) or colloidal silicon dioxide (CAP:II) (4, 23) to prevent vaginal infection with cell-free SIV. Colloidal silicon dioxide meets all the requirements listed in the U.S. Pharmacopeia National Formulary and the European Pharmacopoeia. It is listed as generally recommended as safe and is included in the FDA Inactive Ingredients Guide. It has been used in vaginal preparations (5).

While the SIV female rhesus monkey model probably does not recreate the exact conditions of mucosal transmission of HIV-1 in humans, infection by SIV in monkeys is very similar to HIV-1 and HIV-2 infection in humans (12). The transmission of cell-free SIV across the vaginal mucosa has been well described (1, 2, 12, 14, 32), and therefore the model is particularly useful for evaluating the potency of potential topical microbicides. Here, we describe the successful prevention of infection in 67% of the rhesus monkeys that were treated intravaginally with CAP:II.

The female rhesus monkeys in this study were 10 to 18 years of age and had at least one previous birth with the exception of animal AH37, which was approximately 3 years of age and was nulliparous. The animals were enrolled into either treatment (six animals for each CAP:I and CAP:II) or control (four animals) groups. The rhesus monkeys were housed at the Oregon Regional Primate Research Center, Beaverton; Conserve Research Center, Alice, Tex.; or Yerkes Regional Primate Research Center, Atlanta, Ga. Prior to the study, all animals were tested and determined to be seronegative for antibodies to SIV, type D retrovirus, and simian T-cell lymphotropic virus type 1. All animal care and use procedures conformed to the revised Public Health Service Policy on Humane Care and Use of Laboratory Animals (26). The animals were anesthetized with ketamine intramuscularly prior to all procedures.

The SIVmac251 stock used in this study contained 10⁶ 50% tissue culture infective doses and ~4.3 × 10⁶ SIV RNA copies

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Virus recovery was determined by limiting dilution coculture assay or bulk isolation coculture. The method of determining proviral DNA by PCR.

Two additional control animals were inoculated with virus twice, 3 h later. Similarly, the one virus-negative control and two virus-negative animals were re-treated and inoculated with two vaginal applications of CAP:I and virus. The three CAP:I-treated animals (89D420, H608, and 407L) remained negative for virus isolation. 

As described earlier, three out of six CAP:I-treated monkeys and one of two control monkeys were virus isolation-negative following a single treatment and virus inoculation. These animals were re-treated and inoculated with two vaginal applications of CAP:I and virus. The three CAP:I-treated animals (89D420, H608, and 407L) remained negative for virus isolation, as did four of the six monkeys treated with CAP:II (89C001, P778, 936P, and P407) (Table 1). Virus was recovered from the two additional control animals and the one reinoculated control animal (89D264). Control monkeys had recoverable virus by 2 weeks postinoculation, and virus was consistently recovered through week 12, except in one animal (Table 1). Virus recovery-positive animals had detectable anti-SIV antibodies by 12 weeks postinoculation (Table 2). Two

### TABLE 1. Virus recovery and PCR analysis for proviral DNA following challenge

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal</th>
<th>Virus recovery at wk postchallenge*</th>
<th>No. of animals protected/total</th>
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CAP:I 89D420 0 0 0 0 [-] 1/6
H608 0 0 0 0 [-]
359L + + + + ND
407L 0 0 0 [–]
H671 + + + ND
89D367 + + + ND

CAP:II 89C001 0 0 0 0 [-] 4/6
P778 0 0 0 0 [-]
976W ++ ++ ++ ++ ND
P851 ++ ++ ++ ND
936P 0 0 0 0 [-]
P407 0 0 0 0 [-]

Control 89D264 ++ ++ ++ ++ + 0/4
936P +
59R ++ ++ ++ ++
AH37 ++ ++ ++ ++

* a: Positive results after single treatment and challenge; +, +, positive results after double treatment and challenge; ND, not done; b: negative result.

Symbols in brackets indicate presence (+) or absence (–) of proviral DNA 12 weeks after double treatment and challenge for animals negative for virus isolation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Animal</th>
<th>Absorbance at 405 nm at wk postchallenge</th>
<th>No. of antibody-negative animals/total</th>
</tr>
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<tr>
<td></td>
<td></td>
<td>2</td>
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CAP:I 89D420 0.182 0.221 0.169 0.034 3/6
H608 0.171 0.141 0.151 0.035
359L 0.167 0.455 ND ND
407L 0.157 0.156 0.160 0.043
H671 0.170 0.426 ND ND
89D367 0.170 0.304 ND ND

CAP:II 89C001 0.168 0.055 0.048 0.039 4/6
P778 0.181 0.089 0.013 0.035
976W 0.188 0.236 1.913 1.486
P851 0.193 0.167 0.377 0.172
936P 0.193 0.035 0.036 0.040
P407 0.158 0.066 0.087 0.053

Control 89D264 0.159 0.371 0.853 0.595 0.595 0/4
89D205 0.175 0.466 ND ND
59R 0.156 0.495 1.069 0.347
AH37 0.159 0.452 0.765 0.467

* Results in italics correspond to single treatment and challenge. A whole-virus antibody ELISA was used to evaluate seroconversion to SIV. The historical cutoff for this assay is an optical density of 0.250. ND, not done. Positive responses are indicated in bold.
CAP-I-treated virus isolation-negative and seronegative animals, 89D420 and H608, were positive for proviral DNA at week 12 (Table 1). Animals considered to be protected from virus infection are expected to be negative for virus recovery, not to seroconvert to anti-SIV positivity, and to be negative for proviral DNA. Thus, one of six CAP-I-treated animals and four of six CAP-II-treated animals were protected, while all four control animals from this study became infected. This same virus stock infected six of six animals (C. Miller, personal communication) and five of six animals (3) in studies using the inoculation regimen used in this study.

Transient viremia has been observed in the SIV model after intravenous and mucosal inoculation (17). Transient viremia has been defined as a viremia which occurs briefly and in which the animals remain seronegative. This type of infection generally occurs if animals are inoculated with a very low dose of virus. Thus, the animals in the present study that developed a positive PCR signal in PBMC at a single time point were likely to be transiently viremic because the microbicide treatment greatly reduced the amount of infectious virus in the inoculum. While the significance of transient infections has not been determined, it has been reported that a small number of animals have developed productive infections and progressed to disease with time (12). We have therefore considered the two CAP-I-treated animals that were positive for proviral DNA to be infected, but it is likely that the compound had a role in reducing the infectivity of the challenge inoculum.

The degree of protection observed in this study with CAP:II was similar to that seen in our previous study using 3HP-β-LG, where three of six animals were protected against vaginal transmission of SIV (34), and to the level of protection that has been obtained with N9 (14, 15) in the SIV model. In the present study, two formulations in a glycerol-based cream were tested. The results indicated that formulation of CAP plays a role in the degree of inhibition of infection. The formulations without CAP have been tested in vivo against HSV-2 (4). In these studies, virus shedding was reduced in the animals treated with the colloidal silicon dioxide formulation (formulation II) alone without CAP. The reduction in virus shedding in groups treated with formulation II plus CAP, however, was significantly higher than in those treated with formulation II without CAP (4), indicating that CAP was mainly responsible for the antiviral activity. The contribution of CAP was further evaluated by testing diluted formulation II with and without CAP. After dilution, formulation II without CAP lacked significant anti-HSV activity while formulation II with CAP was still highly effective in preventing vaginal infection of mice with HSV-2 (4). In in vitro assays for anti-HIV-1 activity of pharmaceutical excipients, only CAP and a similar cellulose derivative had activity, while other excipients, including silicon dioxide and glycerol, were inactive (23). Therefore, formulation II without CAP was not evaluated for protective activity in an animal model for vaginal HIV-1 infection.

In CAP:II-treated mice, virus shedding was observed in 11% of the mice compared to 79% of mice treated with CAP-I, indicating that CAP:II has a higher level of antiviral activity against HSV-2 infection (4). In the present study only CAP:II can be considered as effective in preventing transmission of SIV in rhesus monkeys.

The SIV vaginal model of heterosexual HIV-1 transmission is a valuable tool for screening candidate topical microbicides. In order to evaluate the data from studies using small numbers of animals, it is important that all or nearly all of the control animals become infected. In this study, we employed two cycles of treatment and virus inoculation to assess the efficacy of CAP:I and CAP:II. Since transmission of HIV-1 does not necessarily occur from a single exposure in humans, the two inoculations of SIV are appropriate for modeling mucosal infection of humans with HIV-1. Intravaginal treatment by CAP formulated in a glycerol-based cream with colloidal silicon dioxide (CAP:II) was effective in preventing transmission of SIV in 67% of the animals. CAP formulations have been shown to have broad-spectrum activity against viral and bacterial STDs (4, 23). Since CAP is commonly used in the pharmaceutical industry as an enteric film coating material or as a matrix binder for tablets and capsules, the safety of the compound has been extensively documented. In addition, the application of formulated CAP does not appear to cause irritation to the vaginal mucosa in the rabbit model (A. R. Neurath, unpublished).

The data presented here and in other reports from our group suggest that formulated CAP (4, 23) may be a cost-effective, abundant, and safe candidate microbicide with broad-spectrum activity against a range of STDs, including HIV.

This study was supported in part by grants from the Simpson Charitable Trust (A.R.N.).

We thank Cladd Stevens and Beryl Koblin for their contributions.

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