CONCISE COMMUNICATIONS

The Topical Microbicide PRO 2000 Protects against Genital Herpes Infection in a Mouse Model

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Vaginal gel formulations containing the naphthalene sulfonate polymer PRO 2000 are being developed as topical microbicides to protect against infection with sexually transmitted disease (STD) pathogens. A mouse model was used to determine whether PRO 2000 could protect against genital herpes in vivo. Animals received a single intravaginal application of 15 µL of a 10% PRO 2000 aqueous solution or a 4.0% or 0.5% PRO 2000 vaginal gel formulation 20 s prior to intravaginal challenge with 4.0 log10 pfu of herpes simplex virus type 2. Treatment with the 4.0% gel provided complete protection against infection; treatment with the 0.5% gel or 10% solution provided 81% and 80% protection, respectively. Furthermore, the 4% gel provided significant protection even when viral challenge was delayed until 60 min after treatment. This is the first report to show that PRO 2000 can protect against infection with an STD pathogen in vivo.

The incidence of sexually transmitted diseases (STDs) continues to rise at an alarming rate. Currently 5 of the 10 most commonly reported infectious diseases in the United States are sexually transmitted, and an estimated 12 million cases of STDs occur annually [1]. Topical microbicides, which are applied directly to the genital tract on an episodic basis and protect against infection, are an attractive approach to reducing the spread of STDs. The fact that microbicide use can be female initiated (if necessary, without partner consent) has added impetus to the search for safe and effective compounds, because it is recognized that females bear a disproportionate burden of STD infection and are frequently unable to negotiate condom use [1–3]. To date there have been two main approaches to the development of topical microbicides. The first has been to identify detergents or surface active agents that inactivate STD pathogens by disruption of the outer envelope or membrane. The compound that has been most fully evaluated in this regard is the detergent nonoxynol-9 (N-9), which is the active ingredient in many over-the-counter spermicides [4–6].

The second strategy has been to identify compounds that can prevent infection by blocking binding of the pathogen to host cells. The naphthalene sulfonate polymer PRO 2000 (Procept, Cambridge, MA) is an example of this second approach. PRO 2000 was originally found to disrupt early molecular events in the human immunodeficiency virus (HIV) type 1 infection process and to suppress infection by a broad range of HIV isolates [7]. More recent studies have shown that the compound is also active in vitro against two other common STD pathogens: herpes simplex virus type 2 (HSV-2; 50% effective concentration [EC50] < 0.03 µg/mL) and Chlamydia trachomatis (EC50 0.6 µg/mL) [8]. Thus, it has the potential to act as a broad-spectrum microbicide. In addition, PRO 2000 has a number of other characteristics that are desirable in a microbicide. It is straightforward to synthesize, highly water soluble and stable, virtually colorless and odorless, and compatible with latex condoms. Furthermore, two recent phase 1 clinical trials showed that vaginal gel formulations containing ≤4% of PRO 2000 are safe and well tolerated (unpublished data).

In the studies reported here, we examined the ability of PRO 2000 both in solution and in the vaginal gel formulations used in recent clinical trials to act as a topical microbicide against HSV-2 infection in vivo in a mouse model of genital herpes.

Materials and Methods

PRO 2000. PRO 2000 powder was synthesized by the polymerization of 2-naphthalene sulfonic acid and formaldehyde, followed by the selective precipitation of a 5 ± 1 kDa low-polydispersity fraction. The 4.0% and 0.5% (wt/wt) PRO 2000 vaginal gel formulations were prepared by combining PRO 2000 powder in water with 2.0% and 1.35% Carbopol 1382 (B. F. Goodrich, Cleve-
Table 1. Effect of PRO 2000 against genital herpes simplex virus type 2 in mice.

<table>
<thead>
<tr>
<th>Series</th>
<th>PRO 2000 concentration (%)</th>
<th>Vehicle</th>
<th>No. inoculated</th>
<th>No. protected against infection (%)</th>
<th>No. protected against disease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 PBS</td>
<td>15</td>
<td>15</td>
<td>0</td>
<td>1 (7)</td>
</tr>
<tr>
<td></td>
<td>10 PBS</td>
<td>15</td>
<td>12 (80)b</td>
<td>12 (80)b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 Gel</td>
<td>15</td>
<td>2 (13)</td>
<td>2 (13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 Gel</td>
<td>15</td>
<td>15 (100)b</td>
<td>15 (100)b</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.5 Gel</td>
<td>16</td>
<td>13 (81)b</td>
<td>14 (88)b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4 Gel</td>
<td>16</td>
<td>16 (100)b</td>
<td>16 (100)b</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. All mice were treated for ~20 s relative to virus inoculation.

* Animals that did not develop symptoms were defined as infected if virus was isolated from vaginal swabs collected on day 2 after inoculation.

b P < .001 vs. appropriate control by Fisher’s exact test.

land, OH), respectively, 0.05% lactic acid, and trolamine (to pH 2.0). Gel 173 (18) 4 (24).

Results

In the initial study (table 1; series 1), groups of mice were treated intravaginally with 15 μL of a 10% (wt/wt) PRO 2000 solution in sterile PBS or with 15 μL of 4% PRO 2000 gel 20 s prior to virus inoculation. Control animals received the same

volume of sterile PBS or the placebo gel formulation. The incidence of infection was comparable in both control groups (15/15 PBS vs. 13/15 placebo; 28 of 30 animals were infected in total). Subsequently, all but 1 of these mice developed symptoms and died, demonstrating that the placebo gel provided no protection against viral challenge. In contrast, PRO 2000, both in solution in PBS and in the 4% gel formulation, provided significant protection against disease and infection, compared with the appropriate control group (P < .001 each). All 3 PRO 2000–treated animals that became infected were in the group that received the 10% PRO 2000 solution. Consequently, we chose to use the gel formulation in subsequent studies, to further evaluate PRO 2000 as a topical microbicide. In a second study (table 1; series 2), animals treated with the 4% gel 20 s before viral challenge were completely protected. In addition, treatment with a lower dose (0.5%) PRO 2000 gel was also effective, providing substantial but not complete protection against both disease and infection, compared with the placebo gel (P < .001 each).

We next examined the effect of the time of application on efficacy. Table 2 shows that mice treated with the 4% PRO 2000 gel 5 min before viral challenge were completely protected against infection. When the gel was administered 15 min before challenge, 11 of 12 mice were protected, and even when the gel was applied 60 min before challenge we observed both significant protection against infection (P < .01) and development of disease (P < .05).

Discussion

An effective microbicide must be both highly protective and safe even when administered one or more times daily. This safety requirement has led to concerns that frequent application of microbicides containing surface-active agents at concentrations sufficient to inactivate pathogens might also cause damage to the vaginal epithelium and thus, under certain circumstances, actually increase susceptibility to STD infection. Clinical studies with N-9 appear to confirm these concerns, since there is

Table 2. Effect of time of administration on the efficacy of PRO 2000 gel against genital herpes simplex virus type 2 in mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time administereda</th>
<th>No. inoculated</th>
<th>No. protected against infection (%)</th>
<th>No. protected against disease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>−20 s</td>
<td>15</td>
<td>1 (7)</td>
<td>4 (27)</td>
</tr>
<tr>
<td>4% PRO 2000</td>
<td>−5 min</td>
<td>12</td>
<td>12 (100)b</td>
<td>12 (100)</td>
</tr>
<tr>
<td>4% PRO 2000</td>
<td>−15 min</td>
<td>12</td>
<td>11 (92)c</td>
<td>11 (92)c</td>
</tr>
<tr>
<td>4% PRO 2000</td>
<td>−30 min</td>
<td>12</td>
<td>7 (58)d</td>
<td>9 (75)d</td>
</tr>
<tr>
<td>4% PRO 2000</td>
<td>−60 min</td>
<td>12</td>
<td>7 (58)d</td>
<td>9 (75)d</td>
</tr>
</tbody>
</table>

NOTE. All treatment was given in gel.

a Time relative to virus inoculation.

b Animals that did not develop symptoms were defined as infected if virus was isolated from vaginal swabs collected on day 2 after inoculation.

c P < .001 vs. placebo, Fisher’s exact test.

d P < .01 vs. placebo, Fisher’s exact test.

e P < .05 vs. placebo, Fisher’s exact test.
substantial evidence that frequent use can cause inflammation and disruption of the vaginal and cervical epithelium [11, 12]. In addition, while N-9 protected against a number of STD pathogens in animal studies [13, 14], results from clinical trials have been contradictory [4–6]. Thus, there is considerable interest both in the identification of novel compounds with potential as topical microbicides [14–16] and in the development of microbicides, such as PRO 2000, that act by blocking infection rather than by the destruction of the pathogen. It is hoped that such agents, in addition to being highly protective, will not cause cytotoxicity even after frequent use. The results of two recent phase 1 clinical trials provide preliminary evidence that vaginal gels containing ≤4% PRO 2000 are both safe and well tolerated (unpublished data). The results reported here provide the first evidence that PRO 2000 can provide in vivo protection against a recognized STD pathogen, HSV-2.

We show that a single prophylactic application of PRO 2000, either in solution or formulated in a vaginal gel at concentrations used in phase 1 clinical trials, is sufficient to provide significant protection against HSV-2 infection in a mouse model. The 4% vaginal gel formulation was 100% protective when administered shortly before viral challenge and retained good efficacy for at least 60 min. This ability to be effective quickly after application and to maintain protective efficacy for an extended period in the genital tract is an important characteristic for a microbicide, since it is likely that there will be considerable variations between the time of application and exposure to an STD pathogen. On the basis of the observed in vivo effectiveness of PRO 2000 against genital HSV-2 infection, further studies of PRO 2000’s potential as a microbicide are warranted.

Acknowledgment

We thank T. Cunningham for assistance with preparation of the manuscript.

References