Civamide (cis-Capsaicin) for Treatment of Primary or Recurrent Experimental Genital Herpes

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Neuropharmacologic agents able to disrupt normal virus-neuron interactions may provide an alternative strategy for the treatment of herpes simplex virus (HSV) infections. We have previously shown that prophylactic treatment with capsaicin, a natural compound that alters function in sensory neurons, can protect guinea pigs against cutaneous HSV disease, even though the compound has no direct antiviral activity. Here we have examined the ability of civamide, the cis isomer of capsaicin, to interfere with HSV disease. We show that, even when the onset of treatment was delayed until after intravaginal virus challenge, primary genital skin disease severity was significantly reduced. In addition, animals treated during primary infection subsequently experienced a long-lasting reduction in recurrent disease. Civamide treatment during latent infection also significantly reduced recurrent disease, although for a shorter period. Further a single weekly treatment with civamide during latent infection was sufficient to reduce recurrent disease, indicating that an infrequent suppressive maintenance therapy might be possible.

The traditional approach to developing antiviral agents for the control of herpes simplex virus (HSV) infections has been to identify compounds which can selectively interfere with virus replication. An alternative strategy would be to identify compounds which disrupt essential interactions between the virus and host cell. In exploring this approach, we and others have previously reported that the vanilloid capsaicin (8-methyl-N-vanillyl-6-nonenamide) significantly affects the pathogenesis of HSV in animal models (10, 13, 16). Capsaicin has selective neurophysiological and neurochemical effects on sensory neurons with unmyelinated C-fiber processes which include the primary afferents of spinal ganglia (11). These neurons are involved in virus spread and persistence during HSV infection (14). We have previously shown that capsaicin does not affect HSV replication but that treatment of guinea pigs prior to intravaginal HSV type 2 (HSV-2) challenge caused a significant reduction in the severity of primary cutaneous herpetic disease (13, 16). Similarly, when latently infected animals were treated, both spontaneous and UV radiation-induced recurrent infections were reduced (13). Unfortunately, capsaicin is derived from hot peppers, and the initial application is accompanied by an acute burning sensation. This sensation is lost with repeated applications as the neurons become desensitized, forming the basis of the compound’s pain relief characteristics (7, 11). Despite its noxious properties capsaicin has been used as a topical therapy for a number of clinical conditions, such as postherpetic neuralgia, which are associated with chronic pain (3, 4, 6). Civamide is the purified cis isomer of capsaicin and has similar antinociceptive activity (8). It has been evaluated clinically as an intranasal treatment to relieve migraine headache pain (5) and has been shown to be better tolerated than capsaicin in both animals and humans (2, 8). In the studies reported here we have investigated the efficacy of topical civamide treatment for the management of experimental genital HSV infection in guinea pigs.

MATERIALS AND METHODS

Viruses and cells. HSV-2 strain MS was obtained from the American Type Culture Collection (Manassas, Va.). Virus stocks were prepared by culture in low-passage rabbit kidney (RK) cells. Stock virus was maintained frozen (−80°C) until used. Primary RK cells were cultured as previously described (15) and maintained in Eagle’s basal medium supplemented with 10% fetal bovine serum.

Compounds. The 1.25% civamide, 1% capsaicin, and placebo cream formulations used in these studies were prepared and provided by GenDerm Corporation (Lincolnshire, Ill.). For each treatment, animals received 0.2 ml of drug administered intravaginally and topically to the perineal skin. The time of treatment and treatment duration varied with the individual studies and are described below.

Guinea pig model of genital herpes. Female Hartley guinea pigs weighing 300 to 400 g were obtained from Charles River Breeding Laboratories (Wilmington, Mass.) and housed under American Association for the Accreditation of Laboratory Animal Care-approved conditions. For virus inoculation, the vaginal closure membrane was ruptured with a moistened calcium alginate-tipped swab, followed by intravaginal instillation of 5.7 log10 PFU of HSV-2 strain MS into the vagina. On day 1 postinoculation (p.i.) vaginal swab samples were collected from all animals and stored frozen (−80°C) until assayed for the presence of virus on RK cells. All animals were examined daily for evidence of primary genital herpes. Disease severity was quantified by using a lesion score scale described previously (17). Following recovery from primary genital skin disease, animals were observed daily for the development of spontaneous recurrent herpetic lesions on the perineum (18).

Experimental design. (i) Study 1: comparison of capsaicin and civamide treatment of primary genital HSV infection. Antiviral treatment was initiated on day 1 p.i. (24 h after inoculation) and continued twice daily for 10 days. Group 1 (n = 12) was treated with 1% capsaicin cream, group 2 (n = 12) was treated with 1.25% civamide cream, group 3 (n = 12) received a placebo cream, and group 4 (n = 12) consisted of untreated controls.

(ii) Study 2a: effectiveness of different civamide treatment schedules on primary and subsequent recurrent genital HSV-2 infections. For the primary disease treatment portion of the study, animals were assigned to one of five treatment groups (n = 15 each) or served as untreated controls (group 6; n = 60). The 1.25% civamide cream treatment groups and their treatment regimens were as follows: group 1, twice daily for 10 days; group 2, once daily for 10 days; group 3, once daily for 5 days; group 4, once daily for 3 days; and group 5, a single treatment. Treatment was initiated on day 1 p.i.

After all animals had recovered from primary genital skin disease (day 14 p.i.), the untreated control group was divided for the second portion of the study. Twenty animals were maintained as the untreated controls for groups 1 to 5 in order to evaluate the frequency of genital HSV recurrences until day 91 p.i.

(iii) Study 2b: effect of civamide treatment of latently infected animals on the frequency of recurrent genital herpes lesions. Thirty untreated control animals from study 2a were divided into two groups (n = 15 each). All of the animals had experienced symptomatic primary infection, and the levels of severity of the skin disease in the two groups were comparable. One group was treated twice daily with 1.25% civamide cream for 10 days beginning on day 21 p.i., while the other served as untreated controls. All animals were examined daily for recurrent lesions from days 21 to 91 p.i.
Intravaginal and topical treatment beginning 24 h after intravaginal HSV-2 inoculation. BID, twice daily.

Animals that did not develop symptoms were defined as infected if virus was cultured from vaginal swab samples collected on day 1 after virus challenge.

Mean ± standard deviation. Severity is the area under the lesion score versus day curve and was calculated by using only infected animals.

Mean ± standard deviation. Severity is the area under the lesion score versus day curve calculated by using only infected animals.

Intravaginal and topical treatment beginning 24 h after intravaginal HSV-2 inoculation. QD, once a day.

Animals that did not develop symptoms were defined as infected if virus was cultured from vaginal swab samples collected on day 1 after virus challenge.

Mean ± standard deviation. Severity is the area under the lesion score versus day curve calculated by using only infected animals.

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tween days 22 and 63 p.i. \((P < 0.001)\), but this effect was subsequently lost after day 64 p.i. Guinea pigs that received a single treatment weekly for 5 weeks experienced a comparable reduction in recurrent disease. Animals that received only a single treatment weekly for 5 weeks experienced a comparable reduction in recurrent disease. Animals that received only a single treatment weekly for 5 weeks experienced a comparable reduction in recurrent disease.

**DISCUSSION**

Topical treatment with civamide is better tolerated than topical treatment with capsaicin in humans \((2)\). In these studies with guinea pigs topical civamide treatment was also well tolerated. Animals generally displayed evidence of mild discomfort following initial treatments but became rapidly desensitized and subsequently showed no response to further applications of the drug. While civamide is less noxious than capsaicin, it demonstrated similar biological activity in significantly ameliorating experiential primary and recurrent genital HSV-2 infections in guinea pigs \((13, 16)\). In the studies described here treatment with topical civamide or capsaicin begun soon after intravaginal HSV-2 inoculation significantly reduced the severity of the vesiculo-ulcerative genital lesions caused by primary infection. Indeed, a substantial number of the civamide-treated animals developed no genital lesions whatsoever. The effect on primary genital skin disease was seen even when animals received only a single dose of topical civamide.

Civamide treatment of primary infection also had profound effects on the pattern of subsequent recurrent genital herpes. After recovery from the primary infection, civamide-treated guinea pigs experienced significantly fewer spontaneous recurrences throughout a 9-week observation period \((days 15 to 77\ p.i.)\). The effect on recurrent infections was observed even in the group of animals that received only a single topical civamide treatment on the first day after virus challenge. The persistence of the effect on recurrent disease suggests that civamide treatment during the primary infection might affect the establishment of the latent virus infection. Further studies to examine whether civamide treatment of primary infection reduces the magnitude of latent infection are planned.

The effects of topical civamide begun early during primary genital HSV-2 infection were similar to the effects observed with subcutaneously administered capsaicin begun shortly before intravaginal HSV-2 challenge \((13, 16)\). Previous studies have shown that capsaicin lacks antiviral activity in vitro and that prophylactic treatment of guinea pigs, while ameliorating the severity of primary genital HSV-2 infection, does not affect virus replication in the genital tract or in the sensory ganglia \((13)\). However, capsaicin is known to induce numerous biochemical changes in sensory neurons, including depletion of neuropeptides and disruption of axonal transport \((7)\), suggesting that capsaicin and its cis isomer, civamide, may modify genital herpes by interfering with important virus-neuron interactions.

We also examined whether topical civamide was effective in the treatment of recurrent genital herpes when therapy was delayed until after recovery from the primary infection. Civamide treatment of latently infected animals begun on day 21 p.i. significantly reduced the frequency of spontaneous clinically apparent recurrences for several weeks \((days 22 to 63\ p.i.)\).

**TABLE 3.** Effect of civamide treatment during primary infection on recurrent genital herpes

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatmenta</th>
<th>n</th>
<th>No. with recurrences</th>
<th>No. of days recurrences observed between indicated days p.i.b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15–63</td>
</tr>
<tr>
<td>1</td>
<td>BID for 10 days</td>
<td>13</td>
<td>8c</td>
<td>3.1 ± 5.0d</td>
</tr>
<tr>
<td>2</td>
<td>QD for 10 days</td>
<td>13</td>
<td>12</td>
<td>4.4 ± 4.2c</td>
</tr>
<tr>
<td>3</td>
<td>QD for 5 days</td>
<td>15</td>
<td>14</td>
<td>3.1 ± 2.5d</td>
</tr>
<tr>
<td>4</td>
<td>QD for 3 days</td>
<td>15</td>
<td>14</td>
<td>4.5 ± 4.5c</td>
</tr>
<tr>
<td>5</td>
<td>QD for 1 day</td>
<td>13</td>
<td>12</td>
<td>5.5 ± 3.3c</td>
</tr>
<tr>
<td>6</td>
<td>None</td>
<td>20</td>
<td>20</td>
<td>9.8 ± 4.0</td>
</tr>
</tbody>
</table>

\(a\) Intravaginal and topical 1.25% civamide beginning 24 h after intravaginal HSV-2 inoculation. BID, twice daily; QD, once a day.

\(b\) Mean ± standard deviation.

\(c\) \(P < 0.001\) versus no treatment.

\(d\) \(P < 0.05\) versus group 6.

\(e\) \(P < 0.05\) versus group 6.

**TABLE 4.** Effect of civamide treatment of latently infected guinea pigs on recurrent genital herpes

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatmenta</th>
<th>n</th>
<th>No. with recurrences</th>
<th>No. of days recurrences observedb between indicated days p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>22–63</td>
</tr>
<tr>
<td>2b</td>
<td>BID for 10 days</td>
<td>15</td>
<td>15</td>
<td>4.0 ± 2.7c</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>15</td>
<td>15</td>
<td>9.1 ± 3.5</td>
</tr>
<tr>
<td>3</td>
<td>BID for 10 days</td>
<td>15</td>
<td>15</td>
<td>4.2 ± 1.9c</td>
</tr>
<tr>
<td></td>
<td>Once a wk for 5 wk</td>
<td>18</td>
<td>17</td>
<td>3.4 ± 2.1c</td>
</tr>
<tr>
<td></td>
<td>1 dose</td>
<td>18</td>
<td>17</td>
<td>6.5 ± 4.2d</td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>18</td>
<td>18</td>
<td>9.9 ± 5.9</td>
</tr>
</tbody>
</table>

\(a\) Intravaginal and topical 1.25% civamide beginning 21 days after intravaginal HSV-2 inoculation. BID, twice daily.

\(b\) Mean ± standard deviation.

\(c\) \(P < 0.001\) versus no treatment.

\(d\) \(P < 0.05\) versus no treatment.
63 p.i.). While long lasting, the effect was eventually lost, with the treated animals experiencing recurrences at a frequency similar to that for untreated controls late after primary infection (days 64 to 91 p.i.). Remarkably, the long-lasting effect on recurrent infections was observed even in the group of animals that received only a single topical civamide treatment. Other studies have shown that subcutaneous capsaicin treatment of guinea pigs during latent infection can also have a long-lasting but reversible effect on recurrent genital infections (13). Our current findings indicate that a more clinically useful regimen (i.e., single-dose topical treatment) with the less noxious compound (civamide) can also cause a long-lasting reduction in clinically apparent recurrent genital HSV-2 lesions. As with capsaicin, the observation that civamide treatment produced a long-lasting but reversible effect on recurrent genital herpes suggests that the treatment does not destroy latent infected sensory neurons but instead probably acts by inducing a long-term change in neuronal function that profoundly affects virus-neuron interactions important for the pathogenesis of recurrent genital herpes. Possible virus-neuron interactions include reactivation from latency, anterograde virus transport from the ganglia to the periphery, virus assembly at the axon terminal, and virus release from the end of the sensory nerve fiber.

A convenient and well-tolerated topical therapy that reduced symptomatic recurrent genital HSV infections would have obvious clinical benefit, including possible reduction in the risk of virus transmission to a susceptible sexual partner. However, since most transmission probably occurs during periods of asymptomatic virus shedding (1, 9, 21), it will be important to assess whether novel therapies such as civamide also impact unrecognized shedding of HSV from anogenital sites. Chronic daily treatment with traditional nucleoside antiviral drugs such as acyclovir and famciclovir reduces both symptomatic recurrences and asymptomatic virus shedding (12, 19, 20). While it seems reasonable to assume that drugs that act by altering virus-neuron interactions will also affect both clinically apparent and clinically inapparent recurrent infections, it is possible that there are different processes involved in symptomatic disease and asymptomatic shedding. It will be important to establish that amelioration of symptomatic disease is not simply the result of converting a symptomatic recurrence to one that is unrecognized. Such an effect would have very undesirable public health implications.

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