Daily or Weekly Therapy with Resiquimod (R-848) Reduces Genital Recurrences in Herpes Simplex Virus–Infected Guinea Pigs during and after Treatment

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The effect of resiquimod (R-848), an immune-response modifier that is similar to imiquimod, on recurrent herpes simplex virus (HSV) was evaluated using the guinea pig model of genital herpes. Guinea pigs were intravaginally infected with HSV-2 and then were randomized on day 14 to receive nothing or 0.1 mL/kg per dose of subcutaneous resiquimod, given either daily, every other day, or weekly from days 15–35. During a 3-week course of therapy, recurrences in all 3 treated groups were reduced by >80%, compared with the control group. After therapy, recurrences remained significantly (P < .05) decreased in all 3 groups for the next 3 weeks. The group treated weekly developed the fewest recurrences. Significant increases in interleukin-2 levels, produced by incubation of mononuclear cells with HSV-2 antigens, but not in circulating antibody also were detected in the treated groups. Resiquimod treatment may offer significant advantages to present antiviral therapies for the control of recurrent genital herpes.

Herpes simplex virus type 2 (HSV-2) infections are increasing [1]. There are an estimated 300,000 new cases annually in the United States, whereas many more people have unrecognized infection [2]. Seroprevalence estimates suggest that >45 million patients in the United States have genital HSV-2 infections [1]. Many patients with unrecognized disease have recurrent disease that they can be taught to recognize [3]. Others appear to have asymptomatic infections, but even these patients shed HSV-2 from the genital tract [4]. The only currently available therapy for suppressing genital HSV recurrences requires daily administration of antiviral medication [5–7]. Recurrences can be suppressed as long as the patient continues therapy, but return once therapy is discontinued [5].

Resiquimod, also known as R-848 or S-28463, is a member of the imidazoquinoline family of immune-response modifiers [8]. Resiquimod is a more potent analogue of imiquimod (R-837). Imiquimod is approved in the United States as a 5% cream formulation (Aldara) for the treatment of external genital condyloma [9, 10]. Both imiquimod and resiquimod have demonstrated potent antiviral activity in animal models [8, 11]. This activity appears to be mediated predominantly through the induction of cytokines, including interferon (IFN)–α and interleukin (IL)–12 [11–15]. The cytokines produced in response to these agents are largely the result of stimulation of monocytes, macrophages, and dendritic cells [8, 16, 17]. Resiquimod is a more potent inducer of IFN-α and other cytokines than is imiquimod [8].

We have previously shown in guinea pigs that daily topical therapy with imiquimod for 3 weeks can suppress genital HSV-2 recurrences not only for the duration of therapy but also for weeks after therapy is discontinued [14]. We postulated that therapy up-regulated the immune responses that are responsible for controlling recurrent disease. In this report, we have extended these observations to a more potent derivative of imiquimod, resiquimod.

Materials and Methods

Drug. Resiquimod (4-amino-α, 3-dimethyl-2-ethoxymethyl-1H-imidazo[4, 5-c]quinoline-1-ethanol) was provided as the hydrochloride salt by 3M Pharmaceuticals. Resiquimod was administered subcutaneously (between the shoulders) at 0.1 mg/kg per dose after preliminary experiments had suggested this as an optimal dose for prophylaxis of HSV-2 infection in guinea pigs.

Animals. Female Hartley guinea pigs (300–350 g) were obtained from Camm Industries.

Virus. Animals were inoculated with 5.7 log_{10} pfu of MS strain HSV-2 intravaginally, as reported elsewhere [18].

Experimental design. After intravaginal HSV-2 inoculation, animals were examined daily for 14 days to assess the severity of the acute disease [14, 18]. On day 14, animals were randomized into 4 groups so that the severity of the acute disease was comparable among groups [14]. The groups were as follows: group 1, untreated controls (n = 13); group 2, resiquimod administered daily (n = 15);
Table 1. Effect of treatment with different dosing schedules of resiquimod on herpes simplex virus (HSV) recurrences in guinea pigs.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment, days 15–35</th>
<th>Posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days 36–56</td>
<td>Days 57–77</td>
</tr>
<tr>
<td>Control</td>
<td>5.6 ± 4.0</td>
<td>3.7 ± 3.1</td>
</tr>
<tr>
<td>Resiquimod</td>
<td>0.9 ± 1.1a</td>
<td>1.0 ± 1.0b</td>
</tr>
<tr>
<td>Daily</td>
<td>0.9 ± 1.3a</td>
<td>1.4 ± 1.8c</td>
</tr>
<tr>
<td>Every other day</td>
<td>0.8 ± 1.3a</td>
<td>0.9 ± 1.2b</td>
</tr>
<tr>
<td>Weekly</td>
<td>0.8 ± 1.3a</td>
<td>0.9 ± 1.2b</td>
</tr>
</tbody>
</table>

NOTE. Data are mean ± SD of recurrent lesion days. HSV-2–infected guinea pigs were treated with resiquimod daily, every other day, or weekly, from days 15–35, or were not treated (control). Recurrent lesions were evaluated daily from day 15 after HSV-2 inoculation until day 90.

* P < .001 vs. control.
* P < .01 vs. control.
* P < .05 vs. control.

Figure 1. Effect of therapy on cumulative genital recurrences in herpes simplex virus type 2 (HSV-2)–infected guinea pigs. Animals were infected vaginally with HSV-2, were treated with resiquimod either daily, every other day, or weekly for 3 weeks, starting on day (D) 15 after HSV inoculation, and were followed daily for evidence of recurrent genital HSV lesions from day 15 until day 90.
Mean ± SD systemic interferon levels in resiquimod-treated and control guinea pigs. Animals were infected vaginally with herpes simplex virus type 2 and were treated with resiquimod either daily, every other day, or weekly for 3 weeks. Blood samples were obtained before the first dose of drug on day 15 and 8 h after the dose on days 15, 22, 29, and 35. Interferon was measured, as described in Materials and Methods. A, P < .05 vs. control; B, P < .05 vs. daily treatment; C, P < .05 vs. every-other-day treatment.

**Results**

As seen in table 1 and figure 1, all 3 treated groups had significantly (P < .001) fewer recurrences than did the control group over the observation period (67%, 65%, and 75% for the groups receiving treatment daily, every other day, and weekly, respectively). During the 3-week treatment period, recurrences decreased by >80% in each treated group (P < .001 for each group vs. control group). After therapy was discontinued, the number of recurrences remained significantly (P < .05) decreased in all 3 treated groups for the next 3 weeks. The reduction in recurrent disease was somewhat greater for the first 3 weeks after therapy was discontinued, compared with that in the next 3 weeks. In the latter period, only the group given weekly doses of resiquimod developed significantly (P < .05) fewer recurrences than the control group when results were corrected for the multiple comparisons. However, the inability to show significant decreases in recurrences also may be related to the continuing decrease in the recurrence frequency in the control group. The difference in recurrences among treated groups did not approach significance at any interval.

Resiquimod, like imiquimod [8], is a potent inducer of IFN-α. To determine the effects of the different resiquimod regimens on IFN levels, we measured systemic levels of IFN on a weekly basis 8 h after the dose. As seen in figure 2, after animals recovered from acute disease and before therapy (day 15), only background levels of IFN were detected. Eight hours after the first dose, high levels of IFN were detected in all 3 treated groups (P < .001 vs. control group). After 1 week of therapy, IFN levels in the group treated daily had nearly returned to baseline, whereas levels in the other 2 groups remained significantly (P < .05) greater than in the control group and now also significantly (P < .01) greater than the group treated daily. After 2 weeks of therapy, the levels in the group treated every other day also had returned to approximately baseline levels, and only the levels in the group treated weekly remained significantly (P < .05) elevated, compared with those for the control group and now also with the groups treated daily and every other day. On the final day of therapy, it appeared that even the levels in the group treated weekly had begun to decrease, although the IFN levels remained significantly greater (P < .05) than levels in the control and other groups.

To determine the immune parameters that might be associated with the decreased recurrences, we measured HSV-2 antibody and IL-2 levels induced by the incubation of mononuclear cells and HSV-2 antigen. We have reported elsewhere that the best correlate to recurrent disease in guinea pigs was IL-2 levels [20]. HSV-2 antibodies were not significantly increased in any of the treated groups at any time (figure 3). In fact, antibody levels were decreased in the groups treated daily or every other...
day, compared with levels in the control group after treatment (day 35), and the difference between the group treated daily and the control group was significant \( P < .01 \). Furthermore, antibody levels were inversely related to the frequency of treatment, so that after treatment, antibody levels were significantly less in the group treated daily than in the groups treated every other day and weekly, whereas antibody levels in the group treated every other day were significantly less than those in the group treated weekly.

As seen in figure 4A, IL-2 levels were significantly increased in all 3 treated groups on day 49 \( (P < .05 \) for each group vs. control group) and for the groups treated daily and weekly on day 90 \( (P < .05 \) vs. control group). In contrast, IL-2 levels remained similar in all groups after incubation of mononuclear cells with Concanavalin A (figure 4B).

Discussion

We have reported elsewhere that daily treatment with intra-vaginal imiquimod for 3 weeks significantly decreased recurrent HSV-2 disease in guinea pigs and that this decrease correlated with an increase in IL-2 production by mononuclear cells incubated with HSV-2 antigen [14]. In this report, we have extended these observations to show that resiquimod, the more potent analogue of imiquimod, not only significantly reduced recurrences when given daily for 3 weeks but also when given every other day and even when given once weekly for 3 weeks. In fact, the group treated with weekly therapy developed the fewest recurrences for the 3 weeks that animals received therapy and that this significant decrease continued for weeks after therapy was discontinued. This decrease does not appear to be due to circulating IFN, because we have shown elsewhere that IFN induction is short lived and returns to baseline shortly after dosing with this class of immune-response modifiers [14, 19]. Furthermore, in the study reported here, recurrences were decreased in the group receiving therapy daily and every other day, despite the fact that IFN levels had returned to baseline after the first and second weeks of treatment, respectively. The decrease in the IFN response over time is believed to be due to tachyphylaxis. Previous studies also have demonstrated that daily dosing with high doses of this class of immune-response modifiers leads to a down-regulation of IFN and TNF concentrations in mice [15]. This down-regulation is generally not seen when doses are separated by \( \geq 2 \) days. Therefore, regimens of 2–3 times per week probably will maintain IFN concentrations and antiviral responses.

The decrease in recurrences also was not due to the effects of treatment on HSV-2 antibody levels, because antibody levels were actually significantly decreased in 2 of the 3 treated groups. There is no evidence that antibody levels correlate with recurrent disease. Thus, the most likely explanation is that treatment altered the cellular immune response. A decrease in recurrences is probably due to an increased CD4 cell response or, perhaps more specifically, to an increase in the Th1 response so that it is a more dominant response, compared with that in the Th2 component [14, 20, 22]. Other studies clearly have demonstrated the ability of resiquimod to enhance Th1 cytokine release while suppressing Th2 cytokine production [22]. In addition, in vivo studies have demonstrated the ability of resiquimod to enhance
Figure 4. Mean ± SD interleukin (IL)-2 response to herpes simplex virus type 2 (HSV-2) antigen or Concanavalin A (ConA). Animals were infected vaginally with HSV-2 and were treated with resiquimod daily, every other day, or weekly for 3 weeks. Blood samples were obtained on the days indicated, and peripheral blood mononuclear cells were incubated with inactivated HSV-2 antigen (A) or ConA (B) for 48 h. Supernatants then were assessed for IL-2, using a bioassay, as described in Materials and Methods. A, P < .05 vs. control.

antigen-specific IgG2a levels (Th1) while suppressing IgE levels (Th2) in mice [23]. However, an increase in the CD8 cell response also could explain the decreased recurrences [24, 25]. The effects of resiquimod on the HSV CD8 cell response have not been evaluated.

We have been unable to evaluate CD8 cell responses in guinea pigs but have shown in this and other studies of immunomodulators and immunotherapeutic vaccines that the decrease in HSV recurrences is correlated to an increase in IL-2 production from HSV-specific mononuclear cells [14, 20]. Thus, we feel that the effects of this class of immune-response modifiers is probably due to an enhanced HSV-specific Th1 type CD4 cell response.

We postulate that over the 3-week treatment period, the animal’s immune system is exposed to HSV-2 antigen during reactivation of the virus and that, in the presence of antigen, the immune-response modifiers alter the immune response to HSV [19, 20]. This is consistent with the adjuvant activity of imiquimod when used for HSV vaccines in this guinea pig model [26, 27] and the use of resiquimod in a mouse vaccine model [25].

The concept that the frequency of HSV recurrence can be decreased by therapy of limited duration with this class of immune-response modifiers, as shown in the study reported here and elsewhere, recently has been supported in a human trial of topical resiquimod [28]. If verified in larger trials, this novel ther-
therapy for recurrent HSV disease will add to our ability to control HSV infections. To have an impact on the spread of HSV disease, the ability to decrease not only recurrent lesions but also asymptomatic HSV-2 shedding will need to be evaluated.

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References