Topical Resiquimod 0.01% Gel Decreases Herpes Simplex Virus Type 2 Genital Shedding: A Randomized, Controlled Trial

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Background. Resiquimod, an investigational immune response modifier and Toll-like receptor (TLR) 7 and 8 agonist, stimulates production of cytokines that promote an antigen-specific T helper type 1 (Th1)–acquired immune response. In animal models, induction of Th1-specific responses modifies experimental herpes simplex virus (HSV) infection.

Methods. We conducted a randomized, double-blind, vehicle-controlled trial to assess the efficacy of resiquimod 0.01% gel for reducing human anogenital HSV-2 mucosal reactivation. Adults with genital HSV-2 applied resiquimod or vehicle topically to herpes lesions 2 times weekly for 3 weeks and then collected daily anogenital swabs for 60 days for HSV DNA polymerase chain reaction. Recurrences during the subsequent 7 months were treated with study gel. During the final treatment-free 60 days, participants again collected daily swabs to assess shedding.

Results. The median lesion and shedding rates were lower for resiquimod compared with vehicle recipients during the initial sampling period (10% vs. 16% [P = .03] and 10% vs. 17% [P = .08], respectively) and during the final sampling period (3% vs. 22% [P < .001] and 10% vs. 26% [P = .009], respectively). Resiquimod did not influence recurrence length.

Conclusions. These findings suggest that the immunological control of HSV-2 reactivation and lesion clearance may differ and that TLR7 and TLR8 agonists can reduce the frequency of mucosal HSV-2 reactivation.

Genital herpes is a significant public health problem, with 22% of US adults seropositive for herpes simplex virus type 2 (HSV-2) [1]. Currently available antivirals are nucleoside analogues that abrogate viral replication. Although effective and extensively used, they have no effect after treatment is stopped. Several lines of evidence show that the importance of adaptive T cell responses in controlling HSV reactivation [2]. Improving the breadth and magnitude of CD4+ and CD8+ T cell responses to HSV has been shown in both animals and humans to influence viral reactivation and disease.

Resiquimod (R-848), an imidazoquinolinamine, induces production of interferon-α, interleukin (IL)–12, IL-6, IL-8, and tumor necrosis factor–α from dendritic cells, monocytes, and macrophages. Activation of these cells stimulates the innate immune response and leads to a subsequent Th1 cell–mediated immune response [3–6], which can influence immunological control of viral reactivation and clearance.

In a guinea pig model of genital herpes, animals receiving subcutaneous resiquimod demonstrated sig-
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Figure 1. Study time line (A) and enrollment and follow-up (B). SP1, first sampling period; SP2, second sampling period.

significantly fewer recurrent lesion days than did control animals, both during treatment and after treatment discontinuation [7]. One prior human, phase 2, randomized, double-blind, vehiclecontrolled, cohort dose-escalation study in which resiquimod recipients received either 0.05% applied once or twice weekly or 0.01% applied twice or thrice weekly found that those assigned to resiquimod had a longer median time to first recurrence than those assigned to vehicle (169 vs. 57 days; \( P = .006 \)), with the group receiving 0.01% thrice weekly having the longest median time to first recurrence (>195 days) [8]. Subsequent larger trials of topical 0.01% resiquimod conducted concurrently with this trial showed no significant difference in time to first recurrence between the resiquimod- and vehicle-treated groups (T.-C.M., unpublished data). All the above trials used observed genital lesions as the end point for assessing clinical effectiveness. Several studies have shown that most HSV reactivation is subclinical and that daily sampling for HSV DNA is the most accurate and sensitive method for objectively identifying and quantifying HSV reactivation on mucosal surfaces. Thus, we sought to determine whether application of topical resiquimod could influence mucosal HSV-2 reactivation, especially during the period after medication was discontinued when the observed effects would be related to alteration of host immune responses.

SUBJECTS, MATERIALS, AND METHODS

This was a multisite, double-blind, phase 2, randomized, vehicle-controlled trial. Healthy 18–65-year-old HSV-2–seropositive adults were screened, enrolled, and followed between 20 August 2001 and 2 April 2003 at research clinics in Seattle, Washington; Vancouver, British Columbia, Canada; Portland, Oregon; Houston, Texas; and Raleigh, North Carolina. Inclusion criteria included a ≥12-month history of recurrent genital
herpes; ≥4 but <12 recurrences within the past year or, if currently taking suppressive therapy, in the year before beginning suppressive therapy; and >1 recurrence within 3 months before screening. Exclusion criteria included pregnancy, breastfeeding, HIV seropositivity, history of ocular HSV infection, allergy to study drug excipient, suppressive therapy use during feeding, HIV seropositivity, history of ocular HSV infection, before screening. Exclusion criteria included pregnancy, breast-feeding, HIV seropositivity, history of ocular HSV infection, prior resiquimod therapy, hemoglobin level <100,000 platelets/mL, any serum chemistry value of grade 2 or higher, and recent investigational or immunologically active drug therapy or herpes vaccine. The institutional review board at each participating study center approved this study, and all participants gave written informed consent.

The study outline is shown in figure 1. After an initial screening visit, participants entered a 16-week eligibility period during which they were asked to come to the study clinic within 24 h of a recurrence onset (figure 1A and B). Participants with an investigator-verified recurrence were randomized 1:1 within 24 h of recurrence onset to receive topical resiquimod 0.01% gel or vehicle 2 times per week for 3 weeks. On day 22 after randomization, participants started a 60-day treatment-free period of daily home collection of anogenital swabs for HSV DNA polymerase chain reaction (PCR). After completion of this first sampling period, participants entered a 7-month period (weeks 13–43) during which all recurrences were treated with study drug (resiquimod or vehicle) as originally randomized. During weeks 44–52, participants completed a second 60-day treatment-free sampling period. Throughout the entire study symptomatic recurrences were recorded in a participant diary.

The study drug (resiquimod or vehicle gel) was packaged in identical single-dose sachets, each containing 225 mg of gel. Participants applied the entire contents of 1 sachet on each dosing day at bedtime to all visible external herpetic lesions and washed the area 8–10 h later. Doses, applied on days 1, 4, 8, 11, 15, and 18, were applied to healed lesions if lesions were no longer present on the treatment day.

Computer-generated randomization was double-blind, with the study sponsor holding the master code for treatment assignment. Participants were randomized 1:1 in blocks of 6 with stratification by sex.

**Laboratory methods.** HSV serology was performed by Western blot [9]. Anogenital swabs for HSV DNA PCR were obtained by rubbing 2 swabs (held together) across the surface of the penile and perianal areas (in that order) for men and across the posterior cervical/vaginal, vulvar, and perianal areas (in that order) for women. A separate swab was collected from lesions, if present, and placed into a separate vial. All swabs for HSV DNA PCR (performed as described elsewhere [10]) were placed into separate vials containing 1 mL of PCR transport medium and refrigerated. Each PCR run contained negative controls [10], and only samples with >10 copies of HSV DNA per reaction (500 copies of HSV DNA per milliliter of transport medium) were considered positive. If either an anogenital or lesion sample on a given day was positive, the participant was considered to have been shedding that day. Standard clinical assays (complete blood count, comprehensive metabolic panel, urinalysis, and urine pregnancy testing) were used for drug safety evaluation.

**Data analysis.** Efficacy analyses were performed at the University of Washington independently of the study sponsor, and safety analyses were performed by the study sponsor, 3M Pharmaceuticals. Data were analyzed on an intention-to-treat basis using Stata (version 8.0; StataCorp); participants were analyzed with the group to which they were randomized whether or not they applied study drug. The primary end point was the shedding rate (the per-person proportion of swab days with HSV DNA detectable by PCR) during the first sampling period. The median shedding rate in each treatment group was calculated and compared using the Wilcoxon rank-sum test. We used Poisson regression with robust SE estimates to test for a difference between treatment groups in shedding rates during...
Table 2. Shedding and clinical recurrence outcomes.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>First sampling period</th>
<th>Second sampling period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resiquimod (n = 31)</td>
<td>Vehicle (n = 38)</td>
</tr>
<tr>
<td>Shedding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shedding rate, median</td>
<td>10 (10)</td>
<td>17 (16)</td>
</tr>
<tr>
<td>Subclinical shedding rate, median</td>
<td>3 (3)</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Days with shedding/total days sampled (%)</td>
<td>242/1761 (14)</td>
<td>428/2110 (20)</td>
</tr>
<tr>
<td>Clinical recurrences</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lesion rate, median</td>
<td>10 (10)</td>
<td>16 (16)</td>
</tr>
<tr>
<td>Days with lesions/total days sampled (%)</td>
<td>207/1873 (11)</td>
<td>448/2222 (20)</td>
</tr>
<tr>
<td>Time to first recurrence, median, days</td>
<td>41 (41)</td>
<td>28 (28)</td>
</tr>
<tr>
<td>Annualized recurrence rate, median</td>
<td>4.9 (4.9)</td>
<td>9.2 (9.2)</td>
</tr>
<tr>
<td>Duration of recurrence, median, days</td>
<td>3 (3)</td>
<td>5 (5)</td>
</tr>
</tbody>
</table>

NOTE. NA, not applicable.

* Wilcoxon rank-sum test.

b Log-rank test.

the first sampling period after adjustment for differences between groups in baseline characteristics.

Secondary end points included the shedding rate by treatment group during the second sampling period, analyzed as above; the subclinical shedding rate, lesion rate, median annualized recurrence rate, and median recurrence length by treatment group during each of the sampling periods; and the time to first recurrence. A per-person subclinical shedding rate was calculated during each sampling period as the proportion of days without lesions during which HSV DNA was detectable, with differences between groups analyzed using the Wilcoxon rank-sum test. The per-person lesion rate (the proportion of diary days with lesions) was calculated during each sampling period with differences between groups compared using the Wilcoxon rank-sum test. A clinical recurrence of genital herpes was defined as a period of days with lesions preceded and followed by at least 1 day without lesions. For each participant during each sampling period, the total number and average length of clinical recurrences was calculated. Because the length of sampling for each person differed slightly, the total number of clinical recurrences for each person during each sampling period was converted to an annualized recurrence rate by dividing the number of clinical recurrences by the number of sampling days and multiplying by 365. Within each treatment group and sampling period, the median annualized recurrence rate and median individual recurrence length were calculated with differences assessed using the Wilcoxon rank-sum test. Poisson regression with robust SE estimates was used to model the recurrence rate in each treatment group over the entire study. For time under observation, the time from randomization recurrence clear date (the first day without lesions present) to last study visit minus all days with lesions after the first day of each recurrence was used, because a new recurrence cannot be observed during an ongoing recurrent episode.

Kaplan-Meier analysis was used to calculate median time to first recurrence, defined as time from randomization recurrence clear date to start date of the next recurrence, with the log-rank test used to assess difference between groups. If no recurrence occurred before the end of the first sampling period, censoring occurred on the last day of the first sampling period or the last day the patient was seen, if the patient dropped out early. To examine time to healing of the randomization recurrence, defined as the number of days from lesion onset to clear date, we used Kaplan-Meier analysis and the log-rank test to assess difference between groups. We censored participants who were lost to follow-up before a documented clear date at the time of the loss to follow-up.

Power calculation. The primary end point was to compare the effect of resiquimod versus vehicle on HSV shedding rate during the first sampling period. Based on previous data, the vehicle group mean shedding rate was estimated to be 33%, and the overdispersion parameter to be 7.24. Based on 1000 simulated data sets and 100 participants distributed 1:1 to drug versus vehicle, the study would have 69% power to show a 30% reduction in shedding and 93% power to show a 40% reduction in shedding on drug. No interim analyses were planned. In August 2002, the sponsor elected to stop enrollment at 75 participants because of slow enrollment. In early 2003, results from 3 large phase 3 clinical studies of resiquimod using the same dosing regimen used in this study showed no clinical benefit (T.-C.M., unpublished data). At this time, the sponsor decided to prematurely terminate follow-up for our study, reducing the number of participants who completed the second sampling period.
RESULTS

Of 190 persons screened for study eligibility, 142 entered the 16-week eligibility period, and 75 had an investigator-verified recurrence and were randomized (figure 1B). Participants in both groups were similar with the exception that vehicle recipients were more likely than resiquimod recipients to be both HSV-1 and HSV-2 seropositive (table 1). Five resiquimod recipients and 1 vehicle recipient dropped out before starting the first sampling period, including 3 resiquimod recipients who dropped out because of severe or ongoing herpes lesions after 2, 3, and 5 applications of resiquimod; 2 resiquimod recipients who were never documented to have applied any drug and were lost to follow-up; and 1 vehicle recipient who dropped out for a personal reason after 2 drug applications (figure 1B). Among the 31 resiquimod and 38 vehicle recipients who entered the first sampling period, 66 applied all 6 initial doses of study drug, 2 resiquimod recipients applied 5 doses, and 1 applied none.

During the first sampling period, which started 22 days after the initial application of study drug, the rates of clinical reactivation and overall shedding of mucosal HSV-2 tended to be lower among resiquimod compared with vehicle recipients (table 2 and figure 2A and 2C). The proportion of total swab days with shedding was 14% for resiquimod and 20% for vehicle recipients. Using Poisson regression, resiquimod recipients had two-thirds the shedding rate of vehicle recipients (rate ratio, 0.68 [95% confidence interval {CI}, 0.43–1.06]; P = .09). Simultaneous adjustment for baseline HSV-1 serostatus, age, sex, race, and number of recurrences in the year before enrollment did not appreciably change this estimate of effect (data not shown).

Most participants who completed the first but not the second...
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Figure 3. Kaplan-Meier estimates of time to first herpes recurrence (A) and time to clearing of randomization recurrence (B), by treatment group.

The median shedding rate was significantly lower among resiquimod compared with vehicle recipients both during the first sampling period (10% vs. 16%; \( P = .03 \)) (table 2 and figure 2C) and the second (3% vs. 22%; \( P < .001 \)) (table 2 and figure 2D). As measured from the resolution date of the randomization recurrence, resiquimod recipients tended to have a longer time to first recurrence than vehicle recipients (median, 41 vs. 28 days; \( P = .25 \)) (figure 3A). The annualized recurrence rate during the first sampling period among resiquimod recipients was almost half that among vehicle recipients and during the second was significantly lower (table 2). Over the entire study duration, the annualized recurrence rate in the resiquimod group was 30% lower than in the vehicle group (6.5 vs. 9.3 recurrences/year; rate ratio, 0.70 [95% CI, 0.48–1.02]; \( P = .06 \)).

No serious adverse events occurred in resiquimod recipients. The median time to healing of the randomization recurrence was 8 days in both groups (figure 3B). There was no difference between the treatment groups in the number of participants who reported at least 1 adverse event during the initial treatment period (22/36 [61%] resiquimod and 24/39 [62%] vehicle recipients; \( P = 1.0 \)). No clinically meaningful laboratory abnormalities attributable to resiquimod were observed. One resiquimod recipient discontinued treatment because of grade 3 ulceration, defined as ulceration encompassing >10% of the drug application area. However, when the 2 treatment groups were compared, there were no statistically significant differences in the maximum severity of any local skin sign as assessed by either study staff or participants during either the initial treatment cycle or the entire study period (data not shown).

**DISCUSSION**

This study used an objective measurement of HSV reactivation to measure the effect of 0.01% topical resiquimod on mucosal reactivation of HSV-2 in immunocompetent adults. Our data indicate that topical application of this experimental TLR7 and TLR8 agonist reduced subsequent HSV-2 reactivation as measured by reduction in viral shedding for 60 days after stoppage of the medication. Resiquimod’s effect appears to have been primarily mediated through decreasing viral shedding and the number of lesional recurrences observed. There was no measurable effect of resiquimod on the duration of lesions. These
data suggest that the immunological responses influencing HSV-2 mucosal reactivation versus lesion healing may differ. Our data also suggest that in vivo reductions in HSV mucosal shedding rates and hence reduction in transmission may be attainable with the development of safe TLR7 and TLR8 agonists.

TLRs recognize highly conserved molecular structures specific to microbes and initiate signaling events leading to activation of host immunity [11]. As such, TLR agonists hold great promise for treatment of infectious diseases, but the majority of TLR research is still preclinical. By showing decreased HSV shedding among resiquimod compared with vehicle recipients, this is the first human randomized clinical trial to demonstrate decreased viral production resulting from resiquimod application. Prior studies of resiquimod have shown an improvement in clinical outcomes in humans with genital herpes [8] but have not measured viral shedding.

Our findings are consistent with the one prior published randomized clinical trial of topical resiquimod in humans with genital herpes, which found a longer time to first recurrence among persons treated with resiquimod, compared with vehicle, during 6 months of observation after treatment [8]. However, they are in contrast to the unpublished findings of 2 other phase 2 and 3 phase 3 randomized clinical trials of topical resiquimod, which found a similar time to first recurrence and annualized recurrence rate among resiquimod- and vehicle-treated participants (T.-C.M., unpublished data). Interestingly, the present study also failed to show a statistically significant difference in days to first recurrence among the 2 randomized groups, although the shape of the curves suggests a slightly longer time to first recurrence among resiquimod recipients. The median recurrence length was similar and the median annualized recurrence rate shorter among resiquimod recipients, compared with vehicle recipients. Resiquimod may therefore act partially by lengthening the time to first recurrence and partially by spacing out the interval between recurrences. Its effect on recurrence length appears to be minimal. Examining separately time to first recurrence, annualized recurrence rate, and median recurrence length is important for understanding the mechanism of action of a drug. However, lesion rate may be the best clinical end point to use in large phase 3 trials of immune response modifiers because it is a summary of the aforementioned 3 end points and is the most clinically meaningful one.

This trial was stopped early by the sponsor because concurrent larger trials of resiquimod failed to show an effect (T.-C.M., unpublished data). The decision to stop was made before data had been analyzed. In retrospect, premature stopping of this trial was unfortunate, because this underpowered study suggests efficacy rather than more definitively demonstrating it. Perhaps preliminary data analyses should have been done before the decision to stop was made, because these might have argued for continuation to trial completion.

Other limitations of this study besides its small sample size with resultant lack of statistical power include incomplete follow-up and a difference in the proportion of HSV-1-seropositive participants in each treatment group. More participants dropped out of the resiquimod group ($n = 5$) than the vehicle group ($n = 1$) after the initial treatment period but before the first sampling period. Although this loss to follow-up carries with it the possibility of introducing bias, the majority of persons with incomplete follow-up in this trial were lost because of early study termination by the sponsor, which should not have biased the results. By chance the vehicle group had a higher proportion of participants who were HSV-1 seropositive than the resiquimod group. One prior study showed that men who were both HSV-1 and HSV-2 seropositive were more likely to shed HSV-2 than were men with HSV-2 infection only [12]. However, another study in women showed no difference in subclinical shedding rates between women who were only HSV-2 seropositive compared with those who were both HSV-1 and HSV-2 seropositive [13]. In the present study, the baseline difference between the treatment groups in HSV-1 seropositivity is unlikely to explain our findings because shedding rates were higher in the vehicle group even after adjustment for baseline HSV-1 serostatus.

Resiquimod is the first drug to show posttreatment efficacy in human genital herpes. Treatment with an intermittently administered drug that reduces subsequent recurrences and viral shedding via modulation of HSV-specific immunity might be preferable over daily suppressive therapy for some persons with genital herpes. Furthermore, if such treatment could lead to long-term reduction in HSV-2 shedding, it might reduce the risk of genital herpes transmission. Further studies of both resiquimod (perhaps with a more optimized dosing regimen) and similar TLR7 and TLR8 agonists should be conducted.

References

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