ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2008, p. 477–482 Vol. 52, No. 2
0066-4804/08/$08.00+0 doi:10.1128/AAC.01173-07


# Effect of Resiquimod 0.01% Gel on Lesion Healing and Viral Shedding When Applied to Genital Herpes Lesions▽

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Received 5 September 2007/Returned for modification 26 October 2007/Accepted 12 November 2007

**Resiquimod, a Toll-like receptor 7/8 agonist developed as a topical treatment to decrease recurrences of anogenital herpes, induces proinflammatory cytokines that may delay lesion healing. Adults with frequently recurring anogenital herpes were randomized within 24 h of onset of a recurrence to vehicle or resiquimod 0.01% gel two times per week for 3 weeks. Subjects underwent daily lesion assessments and sampling for herpes simplex virus DNA PCR for 21 days or until investigator-determined healing of lesion(s). Eighty-two subjects with a mean age of 39 ± 10.5 years and a median of seven recurrences per year were enrolled in the study. The qualifying recurrence was positive by PCR for herpes simplex virus in 68% of subjects. No difference was observed between the vehicle (39 subjects) and resiquimod (43 subjects) groups with respect to time to healing (median of 7.0 days versus median of 6.5 days, respectively; Cox proportional hazard model ratio of 1.229; 95% confidence interval, 0.778 to 1.942; $P = 0.376$). The distributions of maximum severity scores for any investigator-assessed local skin signs and for subject-assessed local symptoms were similar between treatment groups ($P = 0.807$ and $P = 0.103$, respectively). For subjects with at least one positive PCR result, no difference was observed for time to cessation of viral shedding (median of 7 days versus median of 5 days for vehicle and resiquimod groups, respectively; Cox proportional hazard model ratio of 1.471; 95% confidence interval, 0.786 to 2.754; $P = 0.227$). Application of resiquimod 0.01% two times per week for 3 weeks did not delay the healing of genital herpes lesions or reduce acute viral shedding.**

Infection with herpes simplex virus (HSV) is common in the United States, with adult seroprevalence rates of 57.7% for HSV type 1 (HSV-1) and 17.0% for HSV-2 (25). The ability of HSV to establish neural latency allows it to cause recurrent disease. Oral nucleoside analogs, such as acyclovir, are effective when administered episodically to ameliorate an acute outbreak or chronically to suppress recurrent outbreaks (2, 16, 24). Neither of these strategies, however, affect subsequent recurrences once treatment is discontinued as these agents act directly on viral replication (5–7, 15, 20). Resiquimod (R-848, S-28463), a small molecule related to the Toll-like receptor 7 (TLR7) agonist imiquimod, activates via TLR8 in addition to TLR7 (11, 12). These TLR agonists induce endogenous production of alpha interferon (IFN-α), interleukin-12 (IL-12), IL-6, IL-8, and/or tumor necrosis factor alpha from dendritic cells and other innate immune cells, as well as promote dendritic cell maturation (1, 4, 21, 22). Resiquimod and imiquimod ameliorate the natural course of genital HSV infection in animals, possibly short term through cytokines such as IFN-α that inhibit viral replication and long term through augmentation of HSV-specific cellular immunity (3, 9, 10). Increases in serum cytokines have been observed in humans after oral administration of resiquimod, as well as after topical administration of higher concentrations of resiquimod gel (e.g., 0.25%) (18). In a phase II study, application of resiquimod 0.01 or 0.05% gel to active anogenital herpes lesions prolonged the time to next recurrence compared to vehicle (medians of 169 days versus 57 days, $P = 0.006$, respectively) (19). In contrast to when applied to intact healthy skin, however, application of resiquimod 0.05% gel to active herpes lesions was associated with dose-limiting local inflammation (18, 19). The alteration in the safety profile with herpes lesions may have been a consequence of disruption in skin integrity, increasing percutaneous penetration, and/or the presence of preexisting inflammation. Although resiquimod 0.01% gel was well tolerated when applied to herpes lesions in the phase II study, the number of subjects was small, and subjects were seen only on dosing days (two or three times per week). Therefore, as part of a development program evaluating topical resiquimod to modify the natural history of anogenital herpes, we conducted a phase II, multicenter, randomized, double-blind, vehicle-controlled study with daily evaluations to assess lesion healing and viral shedding, with application of resiquimod 0.01% gel or vehicle applied two times per week for 3 weeks to a recurrence of anogenital herpes.

## MATERIALS AND METHODS

Healthy adults (18 to 65 years old) with a clinical history of anogenital herpes were enrolled at 10 centers in the United States. Inclusion criteria included a ≥12-month history of herpes; ≥4 recurrences within the past year or, if on suppressive therapy, ≥4 per year prior to beginning suppression; and ≥1 recurrence within 3 months prior to screening. Exclusion criteria included pregnancy;

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▽ Published ahead of print on 26 November 2007.

breast-feeding; human immunodeficiency virus seropositivity; active genital infection other than herpes; genital physical abnormalities that might affect safety assessments; a history of ocular HSV infection; allergy to study drug excipients; prior resiquimod therapy; hemoglobin level of ≤94 g/liter; ≤$1.5 \times 10^9$ granulocytes/liter; ≤100,000 platelets/ml; aspartate aminotransferase or alamine aminotransferase of >2.5 times the upper limit of normal; and recent investigational, immunomodulatory, or cytotoxic therapy. The study was approved by an institutional review board at each participating study center, and all participants gave written informed consent.

After a screening visit, subjects entered a 12-week eligibility period. Those presenting with an investigator-verified recurrence within 24 h of subject-determined onset were randomized 1:1 to resiquimod 0.01% gel or vehicle twice per week for 3 weeks. A qualifying recurrence required the presence of a papule, vesicle, or ulcer or erosion. Prodrome- or erythema-only events were not sufficient. Individual lesions ≤5 mm apart were assessed together, while lesions >5 mm apart were assessed individually. A lesion was considered healed if all of the following were true: the skin was smooth and unbroken if previously broken (ulcer or erosion); the skin was flat if previously a papule or vesicle; and if a scab was present, it had fallen off. A new recurrence was defined as the appearance of a lesion(s) starting at least 1 day after complete healing of all previous lesions.

Enrolled subjects were seen daily for 21 days or until healing of the qualifying recurrence for investigator lesion assessments (lesion number, size, and location), application site assessments for local signs (erythema, edema, vesicles, erosion/ulceration, and scabbing) and symptom (pain, burning, numbness/tingling, and pruritis), lesion sampling for PCR, and recording of adverse events and concomitant medication usage. Subjects were seen for end of study on day 22, as well as within 24 h of subject-determined healing if healing had not occurred by day 22. Routine laboratory tests, vital sign measurement, and physical examination were performed on days 1 and 22. Subjects were prohibited from using antiviral, immunomodulatory, and cytotoxic drugs during the study.

Resiquimod 0.01% gel (3M Pharmaceuticals, St. Paul, MN) and vehicle (same as the active formulation except for the resiquimod) were packaged in identical single-dose sachets (225 mg of gel). Subjects applied the entire contents of 1 sachet on each dosing day at bedtime to external anogenital lesion(s). Study drug was washed off after 8 to 10 h. If lesions healed during the 3-week treatment period, study drug was applied to the area of the healed lesions. Treatment assignment was by computer-generated randomization at a ratio of 1:1 (resiquimod to vehicle) within a center in blocks of six with stratification by gender.

**Laboratory methods.** Routine clinical laboratory tests were performed by MDS Pharma Services, Toronto, Ontario, Canada. HSV serology was performed by Children's Hospital and Regional Medical Center, Seattle, WA. Swabs of visible lesions were placed into 1 ml of PCR transport medium and refrigerated until PCR analyses at the University of Washington Molecular Diagnostics Laboratory, Seattle, WA, as described previously (23). Each PCR run contained negative and positive controls. Only samples with >10 copies of HSV DNA/reaction (500 copies of HSV DNA/ml of transport medium) were considered positive. Buffer was added to some samples after spillage during shipment; for these samples, the results were considered indeterminate if below the limits of detection and were not included in the analyses.

**Data analysis.** The primary parameter was time to healing, defined as the number of days between the onset date and the investigator-determined date of complete healing of the lesion(s) of the qualifying genital herpes recurrence. The distribution of time to healing was estimated by using Kaplan-Meier survival methodology. Subjects who discontinued before the qualifying recurrence was healed were censored on the day of discontinuation or, if a date was not available, at the last visit. If the proportional hazards assumption was reasonable, the Cox proportional hazards model was used to assess the treatment effect, considering the following possible covariates: treatment, sex, age, duration of the last recurrence, and HSV-1 serostatus. The proportional hazards assumption in the Cox model was tested based on scaled Schoenfeld residuals (8). If the assumption was not met, the time to healing was to be compared by using the Wilcoxon test, with a sensitivity analysis stratifying for sex.

Time to cessation of shedding was defined as the number of days between the investigator-determined onset date of the qualifying recurrence and the date with the first negative (less than the limits of detection) HSV DNA PCR result with no subsequent positive (any value greater than the limits of detection) results for that recurrence. The distribution of time to the cessation of shedding was estimated as described above. Descriptive statistics were used to summarize the quantitative HSV results ($\log_{10}$ transformed.).

Data were analyzed on an intention-to-treat basis by using SAS (SAS Institute, Inc., Cary, NC). Treatment group differences were compared for age, number of months since herpes genitalis diagnosis, number of recurrences within past 12 months, and duration of last recurrence by analysis of variance. Comparisons for differences by sex, race, use of suppressive therapy within past 12 months, and baseline HSV-1 and -2 serostatus were performed by using the Fisher exact test. Adverse events were coded by using the *Medical Dictionary for Regulatory Activities* and summarized by the preferred term.

**Power calculation.** A sample size of 40 per group would have 98% power to detect a difference of 3 days in mean time to healing between the resiquimod and vehicle groups, assuming a common standard deviation of 3.1 (17). This was estimated by using a two-group *t* test with a 0.05 two-sided significance level (nQuery 5.0; Statistical Solutions, Saugus, MA).

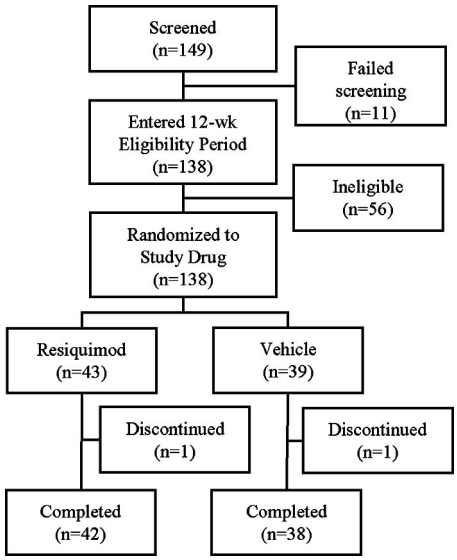


FIG. 1. Disposition of subjects by treatment group.

## RESULTS

**Subjects enrolled.** The first subject was screened on 7 May 2002, and the last subject completed study on 27 January 2003. Of 149 persons screened, 138 entered the 12-week eligibility period. Of these, 82 subjects were randomized (Fig. 1). The most common reasons for nonrandomization were no qualifying recurrence (30 subjects) and study closure after meeting target enrollment (22). Subjects were mostly female (52 of 82 [63%]) and white (66 of 82 [80%]) with a mean age of 39 ± 10.5 years and a median of seven recurrences (range, 4 to 52) per year. Overall, the subjects in the vehicle and resiquimod groups were similar (Table 1); the median total lesion size (in $mm^2$) was slightly higher in the vehicle group, while the median day 1 $\log_{10}$ HSV titer was higher in the resiquimod group (Table 1). One subject (resiquimod) was lost to follow-up immediately after randomization, and one (vehicle) discontinued for personal reasons. One subject (vehicle) was seronegative for HSV-1 and HSV-2 and did not have any positive HSV PCR results. All subjects with a positive HSV PCR result typed as HSV-2; none typed as HSV-1. The median number of doses applied was six for both the vehicle (range, two to six) and the resiquimod (range, four to six) groups.

**Time to healing.** The median times to investigator-confirmed healing of the qualifying recurrence (intent-to-treat) were 7.0 and 6.5 days for the vehicle and resiquimod groups, respectively (Fig. 2). Although the hazard ratio was 1.471 (95% confidence interval, 0.786 to 2.754) for resiquimod treatment after adjustment in the Cox proportional hazard model, there

TABLE 1. Characteristics at baseline among resiquimod and vehicle patients

| Parameter | Patient group | | $P^a$ |
|---|---|---|---|
| | Vehicle ($n$ = 39) | Resiquimod ($n$ = 43) | |
| Mean age in yr (SD) | 39.8 (9.6) | 38.3 (11.3) | 0.536* |
| Sex | | | |
| No. of females (%) | 23 (59) | 29 (67) | 0.495† |
| No. of males (%) | 16 (41) | 14 (33) | |
| Race: no. of subjects (%) | | | |
| White | 32 (82) | 24 (79) | 0.786† |
| Black | 6 (15) | 8 (19) | |
| Other | 1 (3) | 1 (2) | |
| Median time in mo since diagnosis (range) | 130 (9–393) | 102 (7–390) | 0.500* |
| Median time in days since last recurrence (range) | 27 (1–263) | 32 (2–343) | 0.455* |
| Median no. of recurrences per year (range) in past 12 mo | 7 (4–52) | 6 (4–15) | 0.215* |
| Median duration in days (range) of last recurrence | 7 (2–14) | 5 (2–18) | 0.231* |
| No. of subjects (%) with the indicated serostatus | | | |
| HSV-$2^+$, HSV-$1^+$ | 17 (44) | 18 (42) | |
| HSV-$2^+$, HSV-$1^-$ | 18 (46) | 23 (53) | |
| HSV-$2^-$, HSV-$1^+$ | 2 (5) | 2 (5) | |
| HSV-$2^-$, HSV-$1^-$ | 1 (3) | 0 (0) | |
| HSV-2 unknown, HSV-$1^+$ | 1 (3) | 0 (0) | |
| Baseline HSV lesion(s) | | | |
| Median total lesion size in $mm^2$ | 25.0 | 16.0 | 0.118‡ |
| Median no. of lesions (range) | 1 (1–4) | 1 (1–2) | 0.260‡ |
| $Log_{10}$ HSV copies | | | |
| Median (range) | 0 (0–14) | 5 (0–13) | |
| Mean (SD) | 4.5 (5.07) | 5.1 (5.09) | |

[a] The overall $P$ value is given. *, Analysis of variance model with a term for treatment; †, two-sided Fisher exact test (race was grouped as white versus nonwhite); ‡, Wilcoxon rank sum.

was no statistically significant treatment difference ($P$ = 0.376) (Table 2). In addition, no difference was observed in a per-protocol analysis, with median times to healing of 6.7 days for vehicle (32 subjects) and 6.5 (40 subjects) for resiquimod ($P$ = 0.545).

**Time to cessation of HSV shedding.** On day 1, 46% (18 of 31) of vehicle subjects and 49% (21 of 43) of resiquimod subjects had a positive HSV PCR. At any time during the study, 62% (24 of 31) of vehicle subjects and 74% (32 of 43) of resiquimod subjects had at least one positive HSV PCR result. Excluding the subject without laboratory evidence of HSV infection and the subject lost to follow-up, PCR results were not available (no specimen collected, inhibition, processing error, or missed visit) for 48 of 712 (7% overall, 35 of 345 vehicle and 13 of 367 resiquimod) of eligible collection days (until healing or day 22). In addition, for this group there were 29 diluted samples from 20 subjects (10 vehicle and 10 resiquimod subjects) with an indeterminate result. Of the 77 unavailable or indeterminate results, 7 were prior to the cessation of shedding; 1 of these was indeterminate on the day of healing, so the subject (resiquimod) was considered not to have ceased shedding. Twenty-nine of these samples were in 12 subjects (8 vehicle and 4 resiquimod) for which all other results were below the limit of detection; these subjects were not included in the time to cessation of shedding analysis. Thirty-three of these samples were in 14 subjects (7 vehicle and 7 resiquimod) for which there was a time to cessation of shedding calculated.

The median times to cessation of shedding during the qualifying recurrence were 7 and 5 days for the vehicle and resiquimod groups, respectively (Fig. 3). In a "best case" scenario in which positive or negative results were assumed for missing or indeterminate results in order to favor resiquimod, for sub-

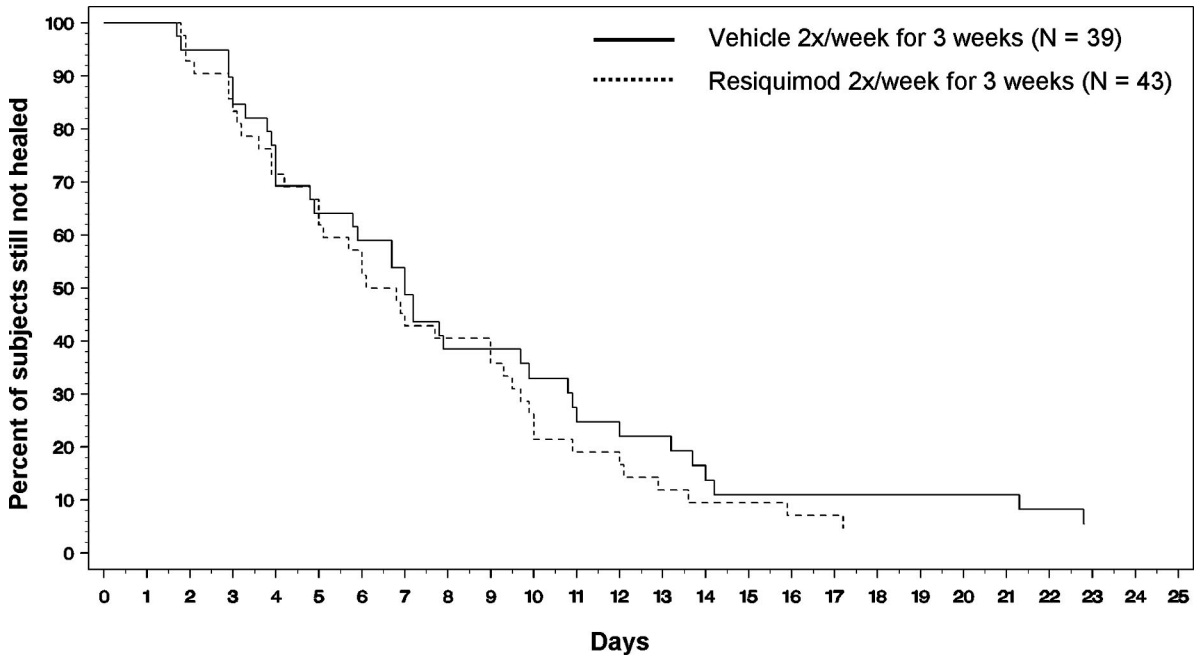


FIG. 2. Kaplan-Meier estimate of time (days) to investigator-confirmed healing of recurrence (intent-to-treat population). The median values were 7 days for vehicle subjects and 6.5 days for resiquimod subjects.

TABLE 2. Summary of Cox proportional hazards model results

| Time period | Parameter[a] | Hazard ratio | 95% Confidence interval | Coefficient | SE | *P* |
|---|---|---|---|---|---|---|
| Time to healing of the investigator-confirmed qualifying recurrence | Treatment | 1.229 | 0.778–1.942 | 0.2065 | 0.23315 | 0.376 |
| | Sex | 0.733 | 0.458–1.174 | –0.3102 | 0.23998 | 0.196 |
| | Age | 1.192 | 0.759–1.872 | 0.1755 | 0.23934 | 0.446 |
| | Duration of last recurrence | 0.691 | 0.429–1.111 | –0.3700 | 0.24262 | 0.127 |
| | Baseline HSV-1 serostatus | 0.790 | 0.492–1.266 | –0.2354 | 0.24041 | 0.327 |
| Time to cessation of HSV shedding of the investigator-confirmed qualifying recurrence | Treatment | 1.471 | 0.786–2.754 | 0.3862 | 0.31987 | 0.227 |
| | Sex | 0.837 | 0.442–1.586 | –0.1776 | 0.32579 | 0.586 |
| | Age | 1.603 | 0.872–2.945 | 0.4718 | 0.31038 | 0.129 |
| | Duration of last recurrence | 1.069 | 0.571–1.999 | 0.0664 | 0.31950 | 0.835 |
| | Baseline HSV-1 serostatus | 1.541 | 0.828–2.866 | 0.4323 | 0.31663 | 0.172 |

[a] Comparisons: treatment, resiquimod versus vehicle; sex, male versus female; age, ≤40 years versus >40 years; duration of last recurrence, ≤6 days versus >6 days; baseline HSV-1 serostatus, positive versus negative.

jects that had at least one positive result (actual or assumed), the median times to cessation of shedding were 8 and 5 days for the vehicle and resiquimod groups, respectively. In a "worst case" scenario, to favor vehicle, the median times to cessation of shedding were 5 and 6 days for the vehicle and resiquimod groups, respectively. Although the hazard ratio was 1.229 (95% confidence interval, 0.778 to 1.942) for resiquimod treatment after adjustments in the Cox proportional hazards model, there was no statistically significant treatment difference ($P = 0.376$) (Table 2b).

**Safety.** During the study, 56% (22 of 39) vehicle and 58% (25 of 43) resiquimod subjects reported at least 1 adverse event ($P = 1.000$). Adverse events reported by ≥10% of subjects in any treatment group are presented in Table 3. For skin and subcutaneous tissue disorders, the differences between treatment groups were marginally significant ($P = 0.056$), with five subjects, all in the resiquimod group, reporting six adverse events. Only two of these subjects reported events within the study drug application site (a vulvar indentation and a skin fissure at the site of former herpes lesions, respectively), and only the skin fissure was considered by an investigator to be possibly related to study drug. The most frequently reported adverse event considered by an investigator to be possibly related to study drug was application site burning (vehicle 13% [5 of 39] versus resiquimod 9% [4 of 43], respectively; $P = 0.730$). During the study, 10% (4 of 39) of the vehicle and 9% (4 of 43) of the resiquimod subjects experienced at least one severe-grade adverse event ($P = 1.000$). The differences in distribution of maximum severity scores for any investigator-assessed local skin signs or for subject-assessed local symptoms were similar ($P = 0.807$ and $P = 0.103$, respectively). The percentages of subjects with any local skin sign graded as moderate or severe (51 and 57% for vehicle and resiquimod subjects, respectively), as well any local symptom graded as

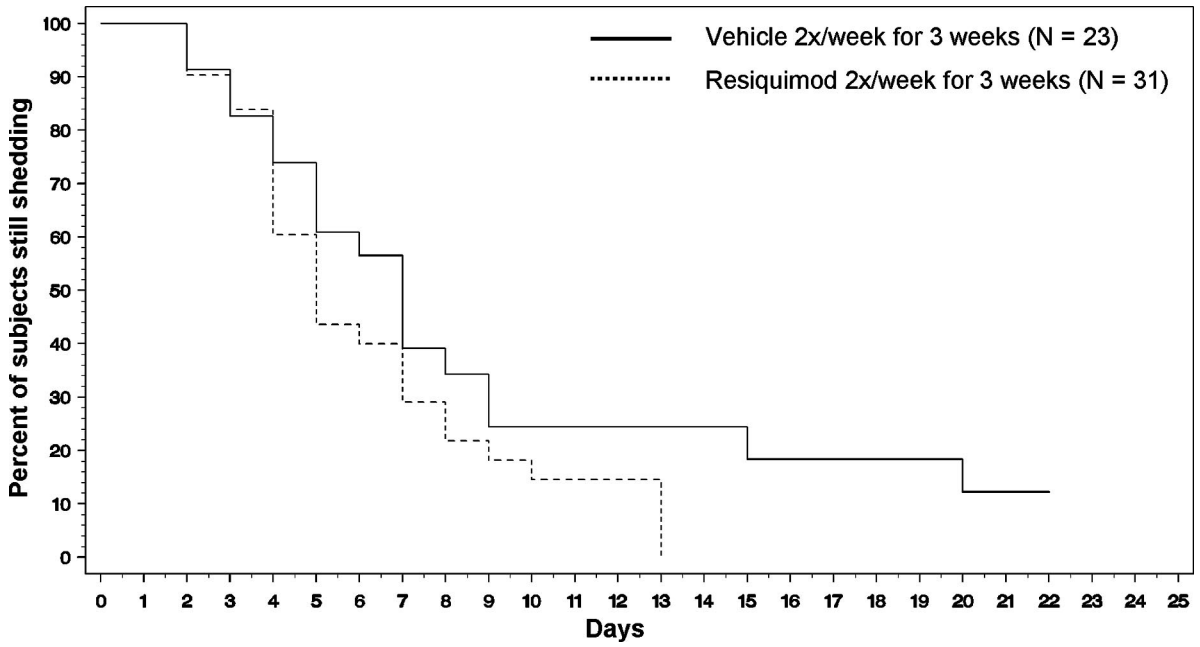


FIG. 3. Kaplan-Meier estimate of time (days) to cessation of viral shedding. Only subjects with at least one positive HSV DNA PCR result were considered in the analysis (23 vehicle and 31 resiquimod subjects). The median values were 7 days for vehicle and 5 days for resiquimod subjects.

TABLE 3. Adverse events, grouped by preferred term, reported by >10% of subjects

| Event(s) | No. of subjects (%) | | $P^a$ |
|---|---|---|---|
| | Vehicle ($n$ = 39) | Resiquimod ($n$ = 43) | |
| At least one adverse event | 22 (56) | 25 (58) | 1.000 |
| General disorders and administrative site conditions | 9 (23) | 5 (12) | 0.241 |
| Application site burning | 5 (13) | 4 (9) | 0.730 |
| Skin and subcutaneous tissue disorders | 0 (0) | 5 (12) | 0.056 |
| Musculoskeletal and connective tissue disorders | 5 (13) | 5 (12) | 1.000 |
| Nervous system disorders | 7 (18) | 11 (26) | 0.437 |
| Headache not otherwise specified | 5 (13) | 7 (16) | 0.760 |
| Respiratory, thoracic, and mediastinal disorders | 1 (3) | 6 (14) | 0.112 |

[a] That is, the overall $P$ value as determined by the two-sided Fisher exact test.

moderate or severe (72 and 57%), were similar in both groups. Although the investigator-confirmed maximum total lesion size was not statistically different between the vehicle and resiquimod groups on day 1 (Table 1), the maximum total lesion size of the qualifying recurrence was greater in the vehicle group over the duration of the study (median of 53.0 versus median 25.0 $mm^2$, respectively; $P = 0.042$ [Wilcoxon rank sum]). There was no difference in median maximum lesion number during the study (median of 1, $P = 0.194$).

## DISCUSSION

In this study, the observed median times to healing were comparable between the vehicle- and resiquimod-treated subjects at 7 and 6.5 days, respectively. The hazard ratio of >1 in the Cox proportional hazard model suggested that the time to healing was shorter with resiquimod treatment, although the confidence interval was wide and spanned 1, indicating that there was no significant difference from the vehicle subjects. In three phase III studies, in which topical resiquimod 0.01% gel was applied to active anogenital herpes lesions using the same dosing regimen, resiquimod was associated with a delay in healing of about 1 week (a median of 9 to 13 days for vehicle groups versus a median of 17 to 20 days for the resiquimod groups) (14). Subjects in the study reported here were seen daily until healing, while subjects were seen, at most, on days 1, 3, 8, 15, and 22 of the first and second treatment courses in the phase III studies. The more frequent assessments in the present study were expected to provide a more precise and accurate estimate of the time to healing, but it may be that a true difference is smaller than could be assessed given the sample size. Another possibility is that although each of these studies used a 1-day healing rule to define a new recurrence, because the start and stop dates of lesions were determined by the subjects in the phase III studies, subjects may have reported lesions to overlap temporally that would have been distinguished as not being part of the qualifying recurrence in the present study. This might be consistent with the longer time to healing in the phase III studies for the vehicle groups compared to that observed in the present study.

The median subject-reported duration of the prior recurrence was slightly longer for the vehicle group than for the resiquimod group, 7 days versus 5 days, which also might have decreased the ability to observe a difference. Although one might speculate that subjects with longer healing times may have chosen not to enroll in the present study because of the daily visits required until healing, the median subject-reported durations of the prior recurrence reported here were similar to those reported in the phase III studies (median of 6.5 to 6.9 days [T. Meng, unpublished data]). Interestingly, in another phase II study focusing on posttreatment HSV shedding that had a similar within-24-h lesion onset entry requirement (versus 36 h in the phase III studies), no differences between the vehicle and resiquimod groups were observed with respect to time to healing (median of 8 days for both) and local adverse events (13). In the present study, the median total lesion size on day 1 was slightly smaller in the resiquimod group (16 $mm^2$) than in the vehicle group (25 $mm^2$), which may have decreased our ability to observe a difference if smaller lesions were more likely to heal faster. The median total lesion size on day 1 was also smaller in the present study than in the phase III studies (30 to 58 $mm^2$ [Meng, unpublished]), which might partially reflect earlier presentation. In the phase III studies there was also an increase in the resiquimod group in the percentage of subjects with moderate or severe local signs or symptoms at the application site (9 to 21%, resiquimod compared to vehicle [Meng, unpublished]). The results described here are again consistent with the time-to-healing results in that there did not appear to be a clear increase in application site reactions.

No treatment difference in the time to cessation of shedding by HSV DNA PCR was observed, but the study did not have sufficient power to determine this endpoint. The ability to detect a difference may have been limited in that 38 and 26% of the vehicle and resiquimod subjects, respectively, did not have any positive samples during the study, and the mean HSV titer was slightly higher in the resiquimod group. The hazard ratio of >1 in the model suggested that the time to cessation of shedding could have been shorter with resiquimod treatment, although the confidence interval was wide and spanned 1, indicating that there was no significant difference from the vehicle. In contrast to the posttreatment effects on overall HSV shedding reported by Mark et al. (13), which would be consistent with an enhanced cell-mediated immune response, the effects observed here would have been expected to be a consequence of induction IFN-α; therefore, the local IFN-α induced may have been insufficient or too late to have meaningful or detectable effects on symptomatic viral shedding.

While 3 phase II studies have suggested that the application of resiquimod to genital herpes lesions might alter the frequency of recurrences posttreatment (13, 19; Meng, unpublished), no statistically significant or clinically meaningful effects on recurrence rates were observed in the phase III studies, resulting in the discontinuation of the development for treating anogenital herpes (14). The variability in observable pharmacologic effects, both with respect to safety and to efficacy, might suggest that the resiquimod 0.01% concentration may have been at the borderline of pharmacologic activity. Exploration of application of higher concentrations of re-

siquimod to herpes lesions could be considered to examine whether more consistent posttreatment effects on recurrences can be achieved, although this is also likely to result in more local inflammation. The effect of differences in the frequency of assessments between any such studies needs to be considered with respect to assessing the time to healing.

## ACKNOWLEDGMENTS

Funding for this study was provided by 3M Pharmaceuticals, St. Paul, MN.

We thank the other study investigators: Keith Aqua (Visions Clinical Research, Palm Springs, FL), Libby Edwards (Mid-Charlotte Dermatology and Research, Charlotte, NC), Raul Gaona (San Antonio, TX), Teresa Jarmul (Boulder Medical Center, Boulder, CO), Terry Klein (Heartland Research Associates, Wichita, KS), Henry Sharata (Madison WI), Dow Stough (Burke Pharmaceutical Research, Hot Springs, AR), and David Whitaker (South County Consultants in Clinical Trials, Wakefield, RI). We are grateful to the staff and patients at all of the study centers for their participation in this study. We also thank Meei-Li Huang (University of Washington Molecular Diagnostics Laboratory, Seattle) for the HSV DNA PCR analyses, An Liu (3M) for statistical assistance, Kim Hart (3M) for study management, Kurt Anderson (3M) for data management and Ronald Hawkinson (3M) for manuscript review.

K.H.F. has been a consultant for 3M and GlaxoSmithKline and has received research support from GlaxoSmithKline, Novartis, Antigenics, and Astellas. D.G.F. has been a consultant for 3M Pharmaceuticals and has received research support from 3M Pharmaceuticals. T.-C.M. and P.L. were employees of 3M.

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