Evaluation of Imiquimod 5% Cream to Modify the Natural History of Herpes Labialis: A Pilot Study

David I. Bernstein,1 Spotswood L. Spruance,2 Sujata S. Arora,3 Jennifer L. Schroeder,3 and Tze-Chiang Meng3

1Children’s Hospital Medical Center Division of Infectious Diseases, Cincinnati, Ohio; 2Division of Infectious Diseases, School of Medicine, University of Utah, Salt Lake City; and 3Medical and Scientific Affairs, 3M Pharmaceuticals, Saint Paul, Minnesota

Background. Imiquimod is currently approved for the treatment of genital warts and has been shown to decrease recurrences of genital herpes in the guinea pig model of genital herpes. Therefore, we evaluated the safety and potential of topical imiquimod to decrease the rate of recurrence in humans with a history of recurrent herpes labialis.

Methods. Forty-seven subjects with recurrent herpes labialis applied imiquimod 5% (n = 30) or vehicle cream (n = 17) to recurrent lesion(s) on days 1, 3, and 5 of the study (day 1 of observation occurred within 48 h after recurrence of lesion). Subjects were seen at the study centers between each dose and 3 days after application of the final dose or until resolution of the lesion.

Results. After application to recurrent lesions, local erythema, edema, scabbing and/or flaking, pain, burning, and maximal lesion size were significantly greater in the imiquimod group than in the vehicle group. The study was terminated early because of severe local adverse events that occurred in 5 recipients of imiquimod. The median time until the next recurrence was, however, increased from 50 days in the vehicle group to 91 days in the imiquimod group (P = .018).

Conclusions. Application of imiquimod 5% cream to herpes labialis lesions was associated with a delay in the time to the first recurrence after treatment, but severe local inflammation occurred in some individuals.

Recurrence herpes labialis, most commonly caused by herpes simplex virus type 1 (HSV-1), affects 20%–40% of adults [1]. Current approved therapies include the nucleoside analogues acyclovir, penciclovir, and valacyclovir, and docosanol (reviewed in [1, 2]). Treatment may be used therapeutically to decrease the duration and severity of ongoing recurrences, or it may be administered daily (prophylactic therapy) to prevent outbreaks [1]. However, the benefits of therapy with the antiviral nucleosides used to treat or suppress HSV infection end when therapy is discontinued [3, 4].

An alternative to antiviral therapy is immunotherapy. Immunotherapy is based on the premise that enhancing immune responses to HSV can reduce the frequency and duration of recurrences [5]. Because cell-mediated immunity appears to play an important role in the control of reactivations, most immunotherapeutic approaches seek to enhance these responses [6]. For example, activity against HSV has been observed for IFN-α in vitro and in vivo [7–9], and more recently, other biologic response modifiers, including imiquimod and resiquimod, have been evaluated as therapy for HSV disease in animal models and in humans [10–12].

Imiquimod is a topically active immune response modifier that induces endogenous production of cytokines, including IFN-α, TNF-α, and IL-12. The activity is mediated primarily from innate immune cells of the monocyte-macrophage lineage [13] through Toll-like receptor 7 (TLR7) [14]. However, imiquimod also affects acquired immunity through enhancement of antigen presentation, maturation of dendritic cells, and indirect induction of IFN-γ [14, 15]. A topical formulation, imiquimod 5% cream (Aldara; 3M Pharmaceuticals), is approved for the treatment of external anogenital warts, actinic keratosis, and superficial basal cell carcinoma [13, 16–18].

In the guinea pig model of recurrent herpes genitalis, administration of imiquimod for 3 weeks resulted not only in a decrease in recurrences during the treatment period but also in a continued reduction in lesion for-
mation after the end of treatment [10]. This posttreatment effect may be mediated by imiquimod acting as an adjuvant for naturally occurring HSV antigen that results from reactivated virus. This would result in enhancement of HSV-specific cell-mediated immunity, as was seen when imiquimod was used as an adjuvant for an HSV vaccine [19–21].

It was hypothesized that application of imiquimod to an active herpes lesion might result in induction of IFN-α and other immune-enhancing cytokines, which, in the presence of HSV antigens, would augment the HSV-specific cell-mediated immunity, thereby delaying subsequent recurrences of herpes labialis. Immune enhancement, however, might also result in an increase in the inflammatory response in the active lesion during application, thereby enhancing the severity of the treated recurrence. Therefore, we conducted a randomized, vehicle-controlled pilot study of application of imiquimod 5% cream to herpes lesions in patients with recurrent herpes labialis to assess the safety and efficacy of this therapy.

MATERIALS AND METHODS

Men and women at least 18 years of age with ≥4 recurrences per year of herpes labialis, who were otherwise in general good health, were enrolled at the University of Utah (Salt Lake City, UT) and at the Cincinnati Children’s Hospital Medical Center (Cincinnati, OH).

Prospective participants presenting within 48 h after the appearance of oral labial herpes lesion(s) were screened by medical history and physical examination of the head and neck, and, when applicable, by pregnancy testing. Qualifying subjects were randomized to receive either imiquimod 5% cream (3M Pharmaceuticals) or a matching vehicle (ratio, 2:1) supplied in individual sachets of 250 mg of cream. A sufficient amount of study drug was applied to cover the herpes labialis lesion(s) and a 1-cm margin around each lesion on days 1, 3, and 5 of the study (day 1 of observation occurred within 48 h after recurrence of lesion); no dose exceeded the contents of 1 sachet. Each dose was applied 30 min before bedtime and left in place for ∼8 h. Subjects were seen at the study centers between each dose to ensure that the next dose could be safely applied and 1–3 days after the final dose. If the lesion had not healed, or if local reactions were grade 2 (moderate) or higher in severity, subjects returned weekly to confirm resolution of the lesions.

Routine hematologic and chemistry samples were obtained on day 1 of the study and at the end-of-treatment visit. A serum sample was obtained for HSV serologic testing on day 1. Swab specimens of the HSV lesion(s) were obtained for viral culture (performed at the respective hospital virology laboratory) on day 1 of the initial recurrence, and at the time of the first recurrence after the end of treatment. Adverse events, including defined local adverse events of specific signs (erythema, edema, vesicles, ulceration, and scabbing and/or flaking) and symptoms (pain, burning, numbness and/or tingling, and pruritis) at the application site, were reported at each visit. Signs and symptoms were scored on a scale of 0–3, corresponding to “none,” “mild,” “moderate,” and “severe,” respectively. Healing of herpes lesions was defined as loss of crust or reepithelialization of ulcers. Upon completion of the treatment period, subjects were observed until their first clinically confirmed recurrence or up to a maximum of 6 months. Concomitant antiviral agents were not permitted during the study. When a new recurrence was detected by the subject, he/she returned to the clinic within 72 h after onset of the episode for clinical evaluation and swabbing of the lesion.

HSV culture and serologic testing. Virus culture was performed in laboratories at each study center according to standard techniques. Lesion swab specimens were obtained and immediately placed into viral transport media, inoculated onto tissue cultures, and observed for at least 7 days. HSV-1 and HSV-2 serologic testing was performed by means of Western blotting at Children’s Hospital (Seattle, WA), in accordance with standard techniques [22].

Statistical analysis. Simulations were performed assuming a γ distribution for time to recurrence for each subject, a 20% dropout rate of patients during the observation period prior to a recurrence, and a median time to recurrence in the vehicle group of 60 days (with a scale parameter of 10 and shape parameter of 6.5). For comparison of days between recurrences in the imiquimod group and the vehicle group, a total sample size of 60 subjects (40 subjects from the imiquimod group and 20 from the vehicle group) provided 68% and 99% power to detect increases in the median time to recurrence to 80 days and to 100 days in the imiquimod group with a Wilcoxon test (α = .05).

Safety and efficacy analyses were performed with the intent-to-treat population, comparing recipients of imiquimod and vehicle. Kaplan-Meier product limit estimates of the time to first recurrence were compared with a Wilcoxon test. The analysis was performed on any subject-reported recurrence (regardless of whether the recurrence was clinically confirmed), and also on recurrences clinically confirmed by the investigative staff. The percent of subjects who had healed (loss of crust or epithelialization) lesion(s) at the end of treatment and who had a recurrence during follow-up were compared using Fisher’s exact test. The maximum severity of each specific sign and symptom, as well as the maximum lesion size during treatment (days 1–7 after the last dose), was compared between groups using the Wilcoxon rank-sum test. All tests were performed at the .05 significance level.

This study was conducted in compliance with the Code of Federal Regulations of the US Food and Drug Administration, 21 CFR Part 56, Institutional Review Boards, and 21 Code of Federal Regulations Part 50, Protection of Human Subjects.
and the ethical principles enunciated in the revised Declaration of Helsinki [23]. The protocol was approved by the Institutional Review Board of each participating institution, and all subjects provided written informed consent.

RESULTS

Forty-eight subjects were screened, and 47 subjects (30 subjects from the imiquimod group and 17 from the vehicle group) of the target 60 subjects were enrolled (figure 1). All subjects were white with a median age of 33 years; 72% were female (table 1). The only statistically significant difference in baseline characteristics between the imiquimod group and the vehicle group was the reported duration of the recurrence prior to entry into the study, with median durations of 8 and 7 days, respectively ($P<.045$). The median number of reported herpes labialis recurrences in the past year was 5 for both groups. Serologic testing for HSV revealed that 38 subjects (81%) were HSV-1 seropositive only, 7 (15%) were HSV-1 and HSV-2 seropositive, and 1 was double seronegative, and 1 subject had a missing sample. Results of HSV culture of the treated recurrence were positive in 77% of subjects in the imiquimod group and in 76% of subjects in the vehicle group.

Safety. During treatment, there was no statistically significant difference between subjects in the imiquimod and vehicle groups in the frequency of adverse events according to body system, including flulike symptoms consistent with systemic cytokine induction or in the safety laboratory tests. However, as seen in table 2, for specifically collected local adverse events, the maximum severities of erythema ($P = .019$), edema ($P = .001$), scabbing and/or flaking ($P = .021$), pain ($P<.001$), and burning ($P = .006$) were greater for imiquimod recipients than for vehicle recipients. There was, however, a higher frequency of moderate or severe erythema already at baseline,

Table 1. Summary of baseline demographic characteristics of subjects with recurrent herpes labialis who were treated with imiquimod or vehicle cream.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vehicle recipients</th>
<th>Imiquimod recipients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>5 (29)</td>
<td>8 (27)</td>
</tr>
<tr>
<td>Female</td>
<td>12 (71)</td>
<td>22 (73)</td>
</tr>
<tr>
<td>Age, median years ± SD</td>
<td>30.6 ± 8.58</td>
<td>35.9 ± 11.50</td>
</tr>
<tr>
<td>White race</td>
<td>17 (100)</td>
<td>30 (100)</td>
</tr>
<tr>
<td>Time since last recurrence, median days</td>
<td>82</td>
<td>84</td>
</tr>
</tbody>
</table>
| Duration of last recurrence, median days | 7 | 8*
| No. of recurrences in the past year | 5 | 5 |

NOTE. Data are no. (%) of subjects, unless indicated otherwise. * $P<.045$, by Wilcoxon rank-sum test.
Table 2. Effect of treatment with vehicle and imiquimod in patients with recurrent herpes labialis, by severity of local adverse event.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle recipients (n = 17)</th>
<th>Imiquimod recipients (n = 30)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local adverse event or symptom, by maximum severity scorec</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythemaa</td>
<td>1 71 53</td>
<td>2 6 23</td>
<td>.189g</td>
</tr>
<tr>
<td>Edema</td>
<td>1 12 40</td>
<td>2 6 23</td>
<td>.001d</td>
</tr>
<tr>
<td>Scabbing and/or flakinga</td>
<td>1 59 23</td>
<td>2 18 33</td>
<td>.021d</td>
</tr>
<tr>
<td>Numbness and/or tinglingb</td>
<td>1 47 23</td>
<td>2 6 23</td>
<td>.114b</td>
</tr>
<tr>
<td>Burningb</td>
<td>1 12 17</td>
<td>2 0 20</td>
<td>.006g</td>
</tr>
<tr>
<td>Pruritus and/or itchingb</td>
<td>1 47 23</td>
<td>2 6 23</td>
<td>.754g</td>
</tr>
<tr>
<td>Maximum lesion size, mm²</td>
<td>Mean ± SD 55.8 ± 67.5</td>
<td>158.8 ± 270.9</td>
<td>.006g</td>
</tr>
<tr>
<td>Median</td>
<td>30.0</td>
<td>84.0</td>
<td></td>
</tr>
<tr>
<td>Healed lesions by end of treatmenta</td>
<td>76 10</td>
<td>.001e</td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data are percentage of patients, unless indicated otherwise.
- a Assessed by investigator.
- b Assessed by subject.
- c 1 = mild, 2 = moderate, and 3 = severe.
- d Wilcoxon rank-sum test.
- e Fisher’s exact test.

prior to dosing in the imiquimod group. The increase in moderate and severe grading appeared as early as prior to the application of dose 2, whereas several other severe grade adverse events occurred a few days after application of dose 3. However, adverse effects were not considered severe enough prior to dose 3 to discontinue dosing for any subject. The maximum lesion size during treatment was also greater in the imiquimod group than in the vehicle group (median, 84 mm² vs. 30 mm²; mean ± SD, 159 ± 271 mm² vs. 56 ± 68 mm²; P = .006). The percentage of subjects who were healed (loss of crust and/or epithelialization) by the end-of-treatment visit (days 6–8) was lower in the imiquimod group than in the vehicle group (10% vs. 76%; P < .001).

The occurrence of severe-grade local adverse events in 5 subjects prompted the early cessation of their enrollment; all 5 subjects were subsequently determined to have received imiquimod. The effect of therapy in 1 of the patients is shown in figure 2. Lesions in the 4 subjects who returned for follow-up visits did not heal until days 19, 19, 29, and 36 of recurrence. Three subjects were treated with antibiotics for presumed bacterial superinfections, 2 received anti-HSV nucleoside analogues, and 3 received anti-inflammatory agents to reduce pain, local swelling, and inflammation. Symptoms experienced by the most severely affected subject included facial pain, erythema, and edema outside of the application site, mild lip numbness, and lymphadenopathy.

Effect on time to next recurrence. The time to the subject-reported first recurrence was longer in the imiquimod group than in the vehicle group (figure 3), with a median time to the first subject-reported recurrence of 91 days and 50 days, respectively (P = .018). Similarly, the median time to the first clinically confirmed recurrence was 109 days for the imiquimod group and 50 days for the vehicle group (P = .081). When evaluated by sex, the time to recurrence was longer for both men and women receiving imiquimod, compared with that for men and women receiving vehicle. The percentage of subjects who reported any recurrence during the follow-up period (up to 6 months) was 73% for the imiquimod group and 94% for the vehicle group (P = .127). Clinically confirmed recurrences were seen in 60% of the imiquimod group and 71% of the vehicle group (P = .540).

Of subjects with clinically confirmed recurrences, lesion symptoms at the time that the subject presented tended to be less severe in imiquimod recipients than in vehicle recipients with respect to pain and burning, but severity was similar with respect to erythema, edema, and vesicles, as well as the size of the recurrent lesions (median size, 30 mm² for the imiquimod group and 27 mm² for the vehicle group). These comparisons are limited because not all subjects presented for their first recurrence and because the recurrence was only assessed at a single visit.

DISCUSSION

The current strategy of managing recurrent herpes labialis primarily focuses on short-term therapy with oral antiherspes nu-
Photograph of a herpes labialis lesion treated with imiquimod obtained on day 10 of recurrence, 5 days after treatment with 3 doses of imiquimod had been completed. The lesion measured 18 × 75 mm and had crusted ulcerations and severe scabbing and flaking reported by the investigator, and moderate pain and mild burning was reported by subject. The lesion healed completely by day 36 without any residual abnormalities.

Figure 3. Kaplan-Meier estimate of time to first subject-reported recurrence after treatment. Subjects with a history of recurrent herpes labialis were randomly assigned to treatment with either imiquimod 5% cream or vehicle. Subjects were evaluated within 48 h after onset of a recurrence and began application to the recurrent lesion. After resolution of the treated recurrence, subjects returned to the study center within 72 h after onset of the next recurrence.

glycosides or over-the-counter medications to shorten the duration and severity of a recurrence; long-term daily suppression is used less frequently [1, 2]. These therapies have no effect on the time to the next recurrence. In contrast, treatment with imiquimod or the more-potent analogue resiquimod decreased posttherapy recurrences of HSV infection in guinea pigs [5, 10]. Furthermore, similar to findings in the study reported here, an extended amount of time to the next subject-reported post-treatment recurrence was also observed in a pilot study of treatment with resiquimod in patients with genital herpes [12]. It is believed that posttherapy effects in guinea pigs and humans are due to enhancement of HSV-specific cell-mediated immunity and not due to effects on innate immunity. The antiviral effect of the induced type 1 IFN, or of IFN-responsive gene products, such as 2',5' oligoadenylate synthetase, would not be expected to have persisted for the duration of the study follow-up. One hypothesis is that application of imiquimod to herpes lesions enhances the HSV-specific immune responses by acting as an adjuvant to the endogenous HSV antigens in the lesion, in a way that is somewhat analogous to a therapeutic vaccine [5, 19].

Application of imiquimod to herpes labialis lesions every other day for 3 doses, however, was not adequately tolerated, and study enrollment was stopped early. Five subjects developed severe-grade local adverse events, prompting termination of enrollment; all 5 subjects had received imiquimod. Later, analyses revealed statistically significant increases in local erythema, edema, scabbing and/or flaking, pain, and burning, as well as increases in lesion size and duration in the imiquimod group, compared with those of the vehicle group. These enhanced local adverse events were consistent with the pharmacologic effect...
of locally induced cytokines, including some with known proinflammatory effects. Thus, it appears that induction of these proinflammatory cytokines exacerbated the preexisting inflammation, leading to significant increases in local reactions rather than reducing acute lesion severity through possible antiviral effects. This is consistent with the limited time that HSV can be recovered from recurrences of HSV infection, the presumed role of inflammation in lesion progression, and the apparent beneficial effects of anti-inflammatory molecules, such as topical corticosteroids, on the disease [24–26].

Qualitatively, the local adverse events were similar to those observed after application of imiquimod 5% cream in other diseases, such as external anogenital warts, basal cell carcinoma, and herpes genitalis [16–18, 27]. The local adverse effects, however, appeared to be more severe after application to herpes labialis lesion(s) than they were after application to herpes genitalis lesion(s), possibly as a result of differences in local penetration due to anatomical differences in skin [27].

In contrast to the results of this study, no posttreatment effect was observed in a study of imiquimod applied to lesions of herpes genitalis [27]. Inconsistent efficacy has also been observed in studies of herpes genitalis treated with resiquimod, a stronger inducer of IL-12 and TNF-α than is imiquimod. Although a reduction in recurrences was observed in 2 phase II studies, the results could not be confirmed in larger studies [12] (personal communication, T. Meng). These differences may be a result in differences in local drug penetration, quantity, and/or quality of the cytokines induced, immunologic responsiveness of the population, dosing strategies, or other as yet undetermined causes. No correlation was observed between subjects having had a severe-grade local adverse event and those having a prolongation in time to recurrence.

In summary, application of imiquimod 5% cream to herpes labialis lesions was associated with a delay in the time to the first recurrence after treatment, but severe local inflammation occurred in some individuals. Alternative dosing regimens or lower concentrations might reduce the risk of severe local adverse effect, but would need to be adequately studied.

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References


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