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Novel Composite Efficacy Measure To Demonstrate the Rationale and Efficacy of Combination Antiviral–Anti-Inflammatory Treatment for Recurrent Herpes Simplex Labialis

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Historically, the primary target for research and treatment of recurrent herpes simplex labialis (HSL) has been limited to inhibiting herpes simplex virus (HSV) replication. Antiviral monotherapy, however, has proven only marginally effective in curtailing the duration and severity of recurrent lesions. Recently, the role of inflammation in the progression and resolution of recurrences has been identified as an additional target. This was evaluated in a randomized study comparing combination topical 5% acyclovir-1% hydrocortisone cream (AHC) with 5% acyclovir alone (AC; in the AHC vehicle) and the vehicle. The efficacy of each topical therapy was evaluated for cumulative lesion size—a novel composite efficacy endpoint incorporating episode duration, lesion area, and proportion of nonulcerative lesions. In that study, cumulative lesion area was significantly decreased with AHC compared with AC (25% decrease; $P < 0.05$) and the vehicle (50% decrease; $P < 0.0001$). As research continues in this arena, cumulative lesion area should be included as a measure of efficacy in clinical trials of recurrent HSL therapies.

The seroprevalence of herpes simplex virus 1 (HSV-1), the HSV type most often associated with herpes simplex labialis (HSL) or cold sores, is estimated to be 58% among immunocompetent Americans 14 to 49 years of age (1) and 31% among children 6 to 13 years of age (2). Approximately 15 to 40% of these seropositive individuals will experience a symptomatic HSL recurrence, the frequency of which depends on genetic susceptibility, immune status, age, site of infection, and viral subtype (HSV-1 versus HSV-2) (3, 4). In most cases, patients with recurrent HSL experience minimal discomfort, pain, and disfigurement, but frequent outbreaks (>5 episodes/year, experienced by approximately 35% of patients with recurrent HSL) are associated with significant impact on physical, emotional, and social well-being (5).

Recurrences of HSL differ from the primary/initial infection with respect to the roles that viral replication and inflammation play in the course of a lesion. In primary infection, lesion development, progression, and resolution are closely correlated with viral replication. In recurrent disease, lesion development and progression depend primarily on the proinflammatory host response (6) (Fig. 1). The central role of inflammation in lesion progression in recurrent disease may explain the limited efficacy of current antiviral monotherapy and suggests a new approach for more effective treatment. The value of one such approach, antiviral–anti-inflammatory combination treatment, has been demonstrated via several endpoints, including cumulative lesion area, a new efficacy endpoint.

DUAL DISEASE PROCESSES IN HERPES SIMPLEX LABIALIS RECURRENCES

Recurrent lesion development: correlations with viral processes. In recurrent HSL, HSV periodically reactivates (by mechanisms that are not completely understood) and moves from the cell body of latently infected sensory neurons toward the epidermis, where the virus begins to replicate. The death of infected cells produces an inflammatory response (discussed below) leading to papule and subsequent vesicle formation, generally within 8 h of the onset of prodromal symptoms (7). Given this short interval and the correlation of lesion area with the neuron sensory field, it is thought that lesions form through coalescence of multiple foci of infection rather than lateral spread within the skin (6). Viral replication is evident as early as the prodromal stage, reaching maximal levels at the vesicle stage and declining rapidly thereafter (8, 9). The level of viral replication, or viral load, correlates with episode severity as measured by lesion area, symptoms, and healing time (8, 10).

The well-recognized importance of HSV replication in lesion pathogenesis has established it as the main target of treatment. Antivirals are effective in shortening episode duration in primary HSV infections; however, in recurrent HSL, topical treatments have only moderate effect on lesion healing time and rarely any effect on lesion area or the proportion of nonulcerative lesions (recurrences that do not progress beyond the papule stage, sometimes referred to as macular, papular, or aborted lesions) (11, 12). Based on results from clinical trials, the benefit of topical antiviral therapy on episode duration in recurrent HSL appears to be limited to about 10 to 15% (11, 12) despite a robust effect on viral replication (13). This may be due in part to the timing of viral replication and treatment initiation. Since viral replication is rapidly controlled (1 to 2 days) by host immune responses (8), if the antiviral drug is applied after viral replication reaches its maximum, there is little opportunity for the drug to have a significant effect, as demonstrated by the differences in efficacy observed when antivirals are initiated early (during the prodrome or erythema stages) versus late (during the vesicle or later stages) (14–18).

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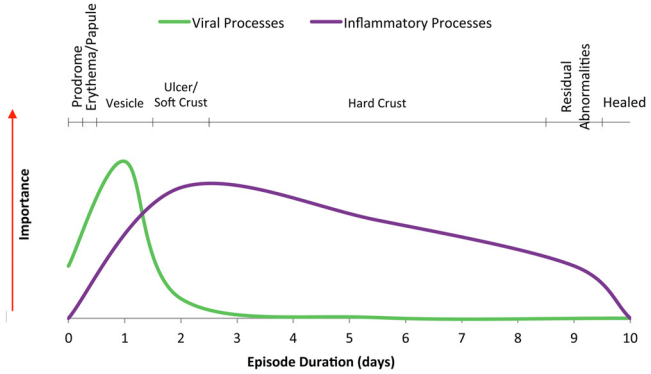

FIG 1 Ulcerative recurrent herpes simplex labialis lesions. Conceptualization of relative contributions of viral and inflammatory processes over time. Episode stages and durations were based on information contained in reference 6.

Recurrent lesion progression and resolution: correlations with inflammatory processes. The importance of robust HSV-specific, cell-mediated immunity to the maintenance of HSV latency has long been appreciated. More recently, the contribution of proinflammatory processes to the progression and resolution of HSL recurrences has been established (19–24). Inflammatory infiltrates are apparent soon after the onset of symptoms (even in those with no visible lesions) and persist during vesicle and crust formation (19–21). In addition, there is evidence that interferon (IFN) and/or other proinflammatory mediators play a role in stimulating cellular responses and preventing lesion area enlargement (23). Histopathologically, vesicles form when HSV-infected cells lyse, inducing cytokine and chemokine production that leads to inflammatory infiltrates of neutrophils, monocytes, and T lymphocytes that extend through the epidermis and basement membrane to the dermis (19–22). In later lesion stages (i.e., "crusted" lesions), eosinophilic infiltration is apparent (19).

Based on the observation that peak interferon levels correlate with the time to the next recurrence (24), Bernstein et al. (25) tested the effects of topical imiquimod, a Toll-like receptor 7 agonist that induces interferon and other cytokines, in patients with recurrent disease. They found that imiquimod, compared with the vehicle, significantly delayed the time to the next recurrence (50 versus 91 days; $P = 0.018$). However, the enhanced immune reaction also caused a significant increase in cutaneous lesion symptoms at the time of application (burning, erythema, edema, scabbing and/or flaking, pain, and burning; $P < 0.05$ for all) and enlarged the maximum lesion area (median, 84 versus 30 mm^2; $P = 0.006$), demonstrating the important contribution of cellular immune responses to lesion progression. In contrast, when local cellular responses were suppressed using oral levamisole, patients experienced dose-dependent reductions in lesion pain (correlation coefficient [r] = −0.34; $P = 0.033$) and duration ($r = -0.32$; $P = 0.051$) but increased recurrence frequency ($r = 0.43$; $P = 0.007$) compared with patients receiving the placebo (26).

MOLECULAR MECHANISMS IN THE NATURAL HISTORY OF RECURRENT HERPES SIMPLEX LABIALIS

After reactivation, HSV travels down the nerve to infect and replicate within keratinocytes (27) (Fig. 2). This results in upregulation and secretion of β-chemokines, interleukins, and adhesion molecules (via NF-κB activation), which attract monocytes, neutrophils, and $CD4^+$ T cells to the site of infection. Resident dendritic cells take up HSV antigens and migrate to the draining lymph node to present antigen to specific central memory cells. Antigen presentation also stimulates $CD4^+$ lymphocytes at the infection site to release IFN-γ and dendritic cells to release interleukin 12 (IL-12). The IFN-γ restores major histocompatibility complex (MHC) class I and II expression in infected keratinocytes and stimulates inducible nitric oxide synthase (iNOS) expression; both IFN-γ and IL-12 activate $CD4^+$ T lymphocyte cytotoxicity.

In addition to any virus-induced cell lysis, the actions of monocytes, neutrophils, and cytotoxic T cells may also result in virus-infected keratinocyte lysis, releasing viral particles along with cellular contents, which causes erythema and edema. Proinflammatory cytokines released from keratinocytes and inflammatory cells (via iNOS) along with prostaglandins (via NF-κB-induced expression of cyclooxygenase 2 [COX2]) dilate blood vessels, leading to greater fluid accumulation and inflammatory cell migration (27). Eventually, these fluids and inflammatory cells coalesce into a vesicle, which, as more and more cells lyse, ruptures to form an ulcer. At this (ulcer) stage, neutrophil infiltration increases as a result of increased IL-8 and E-selectin expression, while $CD8^+$ cells that are local or have migrated from the circulation (due to increased E-

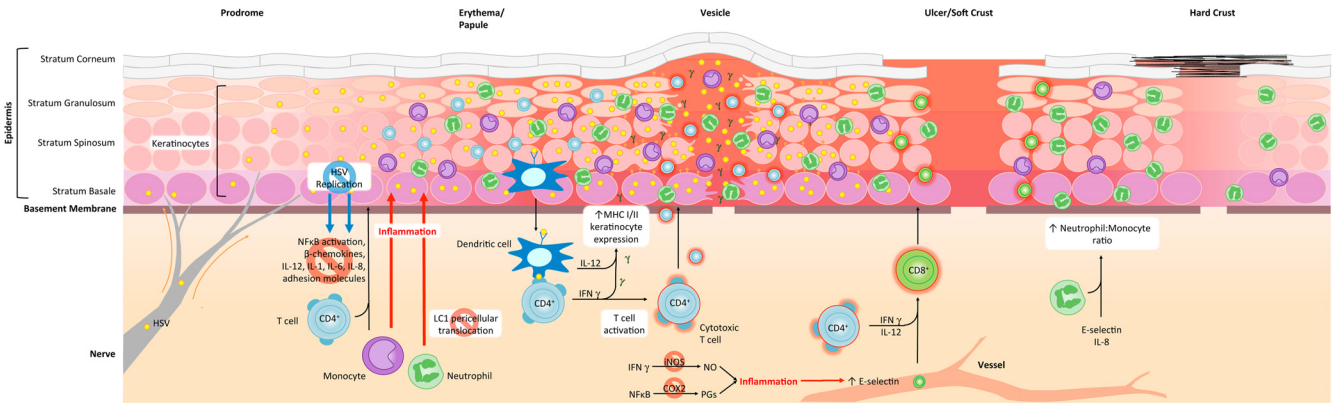

FIG 2 Recurrent herpes simplex labialis lesions. Viral and inflammatory processes and antiviral and corticosteroid targets. COX2, cyclooxygenase 2; HSV, herpes simplex virus; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; LC1, lipocortin 1; MHC, major histocompatibility complex; NF, nuclear factor; NO, nitric oxide; PGs, prostaglandins; red ⊘, inhibited by corticosteroids; blue ⊘, inhibited by antivirals.

selectin expression) are activated by IFN-γ and IL-12 (19, 27). Lesion resolution is characterized by fewer and fewer inflammatory cell infiltrates until the wound heals and the skin returns to normal.

TOWARD MORE EFFECTIVE TREATMENTS FOR RECURRENT HERPES SIMPLEX LABIALIS

Given the contributions of both of the viral and inflammatory processes depicted in Fig. 2, several authors have proposed a role for immunomodulators in mitigating the clinical impact of recurrent HSL (12, 28, 29) by limiting lesion area enlargement and/or and preventing ulcerative lesions by reducing edema, protease activity, and/or inflammation. Using a newly developed mouse model, Harmenberg et al. (30) tested various antiviral-immunomodulator combinations (including corticosteroids and NSAIDs) and found that combinations with corticosteroids (especially hydrocortisone) were most effective at reducing lesion duration and severity. The value of corticosteroids in combination with an antiinfective agent has been demonstrated in the management of herpetic stromal keratitis (31, 32), bacterial meningitis (33), *Pneumocystis jirovecii* (formerly *carinii*) pneumonia (34, 35), herpes zoster (36), and others (37).

These potential benefits are based on the actions of corticosteroids in the skin, as depicted in Fig. 2, namely, inhibition of the expression, release, and/or pericellular translocation of proinflammatory mediators, including lipocortin 1 (LC1), and thereby reduction of neutrophil infiltration and proinflammatory cytokine expression (including the expression of IL-6 and IL-8). These effects occur directly via glucocorticoid response elements or indirectly via inactivation of various transcription factors (38). Corticosteroids also reduce vasodilation, inflammation, and oxidative damage within the skin by inhibiting the cytokine-induced expression of iNOS and thereby reducing NO production. In addition, corticosteroids can interrupt NF-κB-stimulated inflammatory cytokine and AP-1 adhesion molecule expression and reduce COX2 expression and prostaglandin production. Corticosteroids can also block prostaglandin production via LC1-mediated effects on phospholipase A2.

Ultimately, the efficacy of any treatment will depend on its dermal penetration. Recently, a new topical formulation of acyclovir with better dermal penetration showed improved antiviral efficacy in a randomized clinical trial (18). In addition, adding a corticosteroid to an antiviral may have additive or synergistic effects, including enhanced effective dermal concentration of the antiviral component via steroid-mediated vasoconstriction. In a randomized trial of combination oral famciclovir and topical fluocinonide compared with famciclovir alone, combination therapy reduced lesion healing time to normal skin (median, 5.9 versus 8.9 days; $P = 0.06$), reduced maximum lesion area (median, 48 versus 162 mm^2; $P = 0.02$), and increased the proportion of nonulcerative lesions (41 versus 8%; $P = 0.09$) (39).

IMPROVING UPON TRADITIONAL MEASURES OF EFFICACY IN RECURRENT HERPES SIMPLEX LABIALIS

Historically, episode duration and the proportion of nonulcerative lesions have been the main endpoints used in trials of antiviral treatments; however, these endpoints have limitations, and several alternative measures have been suggested. For example, Spruance et al. (14) suggested using the durations of the most painful and disfiguring lesion stages (vesicle, ulcer, and hard crust). They showed that the combined durations of these stages was reduced by 43% ($P < 0.001$) by idoxuridine among patients with ulcerative recurrences, whereas total healing time was unaffected, thereby demonstrating that antiviral treatment can reduce the duration of the most bothersome stages of HSL lesions even if total healing time is unchanged. Similar endpoints have been utilized by Evans et al. (40) and Crane et al. (41). However, lesion stage duration endpoints do not capture the ability of an antiviral to prevent ulcerative lesions, which may be the most important goal of HSL therapy.

Several studies have addressed this by assigning nonulcerative lesions a value of zero for episode duration and lesion area (values can be set to zero because, by definition, nonulcerative lesions have zero durations of the vesicle, ulcer, and crust stages [42]). For example, in combined data from two randomized trials, high-dose oral valacyclovir increased the proportion of nonulcerative recurrences by 6 to 7% ($P < 0.05$) over the placebo (42). When this effect was accounted for in the calculation of episode duration (until loss of the hard crust), the apparent efficacy of oral valacyclovir versus the placebo increased from a 21% ($P < 0.001$) reduction in episode duration (nonulcerative lesions excluded) to a 31% ($P < 0.001$) reduction in episode duration (nonulcerative lesions included). In another randomized trial, when only ulcerative lesions were assessed, topical (via iontophoresis) acyclovir reduced episode duration (until loss of the hard crust) by 26 h compared with the placebo (139 versus 165 h; $P = 0.03$). However, when aborted lesions were included, the difference in median episode duration was even more pronounced (35-h difference [113 versus 148 h]; $P = 0.02$).

Rodu et al. (43) has argued that a better measure may be efficacy in containing a lesion (measured by lesion area) rather than ability to alter the repair process (episode duration). To this end, they devised a multistep method of measuring lesion area and compared values at days 2, 3, 4, and 5. However, because they chose to compare lesion areas by day, strict enrollment criteria were needed so that baseline lesion areas were as homogenous as possible. When they tested the efficacy of a combination of tannic, boric, and salicylic acids in patients with ulcerative lesions, they showed significant reductions in lesion area of 42.6 to 53.4% over the placebo on days 2 to 5.

A more practical approach may be to compare cumulative lesion areas over time (with nonulcerative lesions given a zero value), which reduces the need for strict enrollment criteria. By including nonulcerative lesions, this composite measure would include all clinically important features—lesion area, time to healing, and prevention of ulcerative lesions—and would be a measure of overall treatment benefit. An added advantage of cumulative lesion area is that it is a serial measure, thus quantifying lesion area and duration while giving greater weight to days with ulcer or crust compared to days with redness or tingling.

Cumulative lesion area may be particularly useful when a treatment or combination of treatments targets multiple aspects of the disease process. Hull et al. (44) used such a parameter in a clinical study of combination treatment, although similar measures have been used previously in clinical trials of antiviral monotherapy (41, 45) and in animal studies (46). By adding lesion areas for each day of the episode (until loss of the hard crust), using a value of zero for nonulcerative lesions, they calculated the cumulative lesion area and compared treatment effects. The data demonstrated that a combination of oral valacyclovir and topical clobetasol,

TABLE 1 Efficacy endpoints in study 609-04[c]

Parameter	AHC ($n = 601$)	AC ($n = 610$)	Vehicle ($n = 232$)	% difference[a]		
				AHC vs AC	AC vs vehicle	AHC vs vehicle
Proportion of nonulcerative recurrences of all lesions (%)	42	35	26	**20***	**35***	**62****
Median episode duration (until loss of hard crust; days)						
Ulcerative lesions	5	5	6	0	**16†**	**16†**
All lesions[b]	3	4	5	**25†**	**20††**	**40††**
Mean maximum lesion area (mm^2)						
Ulcerative lesions	40.7	42.3	49.5	3.8	14.5	17.8
All lesions[b]	23.5	27.3	36.6	13.9	**25.4‡**	**35.8‡**
Mean cumulative lesion area (mm^2)						
Ulcerative lesions	134.5	162.6	209.0	17.3	22.2	**35.6‡**
All lesions[b]	77.6	104.9	154.7	**26.0‡**	**32.2‡**	**49.8‡‡**

[a] Bold text indicates a significant difference. *, $P < 0.05$; **, $P < 0.0001$ via the chi-square test; †, $P < 0.05$; ††, $P < 0.0001$ via the log rank test; ‡, $P < 0.05$, ‡‡ $P < 0.001$ via the two-sample *t* test.
[b] Nonulcerative lesion values were set to zero.
[c] Values are from the intent-to-treat population. AC, 5% acyclovir cream; AHC, 5% acyclovir-1% hydrocortisone cream.

compared with the placebo, had a dramatic effect on mean cumulative lesion area (23 versus 193 mm^2; $P = 0.001$).

APPLICATION OF CUMULATIVE LESION AREA AS A MEASURE OF COMBINATION TOPICAL THERAPY EFFICACY

Recently, the cumulative lesion area endpoint was used in a randomized, double-blind study to evaluate the efficacy of the combination 5% acyclovir-1% hydrocortisone cream (AHC) (Xerese; Valeant Pharmaceuticals North America LLC, Bridgewater, NJ) in patients with recurrent HSL (study 609-04; NCT00361881) (18). The combination AHC, which was approved by the U.S. FDA in 2009 for the early treatment of recurrent HSL to reduce the likelihood of ulcerative lesions and shorten lesion healing time in patients ≥12 years of age (47), has been shown to be safe and effective (18, 40) without evidence of an increased risk of developing acyclovir resistance (18, 48). In the 609-04 study, AHC was shown to prevent lesion progression (the proportion of nonulcerative lesions), shorten episode duration, and decrease maximum lesion area compared with 5% acyclovir cream (in the AHC vehicle, AC; significantly in some cases) and the vehicle (significantly in most cases) (Table 1). These effects contributed to a reduction in cumulative lesion area of just over 25% with AHC compared with AC ($P < 0.05$) and a nearly 50% reduction compared with the vehicle ($P < 0.0001$). These results illustrate the additive nature of cumulative lesion area in capturing the full morbidity, or total burden, of an HSL recurrence. In other words, cumulative lesion area for "all lesions" quantifies efficacy in aborting recurrent HSL lesions and, failing that, in reducing ulcerative lesion area and episode duration.

CONCLUSIONS

Antiviral monotherapy has been the primary focus of research and management of recurrent HSL for many years, but recently, there has been a greater appreciation of the contribution of inflammatory mechanisms to recurrent HSL lesion progression and resolution. This led to investigations into the use of antiviral–anti-inflammatory combinations, which have been shown to be more effective than antivirals alone (18, 39, 40, 44). Because HSL therapy, especially combination treatments, can have multiple effects—prevention of lesion progression, reduced lesion area, shorter episode duration—composite endpoints may be the most appropriate measures of their total efficacy. Cumulative lesion area is a novel composite endpoint that accounts for all aspects of lesion severity. In a recent clinical trial (18), cumulative lesion area was used to demonstrate the overall efficacy of AHC in reducing overall lesion burden by 50% ($P < 0.0001$), an improvement over the efficacy of AC (over 25% reduction). All other efficacy measures captured only a portion of the improved efficacy of AHC. This lends support to the use of cumulative lesion area in future clinical trials of HSL therapy.

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