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Mini-Review

Topical treatment of cutaneous herpes simplex virus infections

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Summary

Topical antiviral chemotherapy has a number of potential advantages over systemic drug delivery for the treatment of cutaneous herpes simplex virus infections, including convenience; higher target tissue drug levels and greater efficacy; and specific targeting of the drug to the site of infection, with reduced cost and reduced exposure of the remainder of the body to drug side effects. Realization of these possibilities has been slow in part because of the paucity of 'active' topical drug formulations with effective penetration-enhancing agents and a technical barrier – our failure as yet to measure drug levels in the epidermis, which could guide formulation development. Recent success with a topical treatment for herpes simplex labialis should stimulate continued laboratory and clinical research in this field.

Herpes simplex virus; Topical drug formulation; Clinical trial; Antiviral therapy

Introduction

Successful chemotherapy of herpes simplex virus (HSV) infections has been achieved by the intravenous, oral and topical routes of drug administration. When infection is limited to the skin, topical therapy should be considered because of convenience; reduced systemic exposure to drug side effects; and the potential, with the use of skin penetration enhancers, of higher concentrations of antiviral agent at the site of viral replication than might be achieved by systemic drug delivery. Human HSV infections where topical treatment should be considered

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include herpes genitalis, herpes labialis, herpetic whitlow and herpes keratitis.

Frequently recurrent herpes genitalis can be successfully managed by the long-term prophylactic peroral administration of acyclovir (ACV) (Straus et al., 1984; Douglas et al., 1984). However, many patients are not candidates for prophylactic ACV. For those patients who need treatment of individual episodes, only small benefits have been accomplished to date with peroral or topical ACV (Corey et al., 1982; Fiddian et al., 1983b; Kinghorn et al., 1983; Luby et al., 1984; Mattison et al., 1988; Nilsen et al., 1982; Reichman et al., 1983; Reichman et al., 1984; Ruhnek-Forsbeck et al., 1985). Both the topical and peroral routes of antiviral drug treatment of herpes genitalis should continue to be explored to provide a satisfactory alternative for patients not on drug prophylaxis.

Antiviral drug treatment of primary HSV infections or infections in immunocompromised patients has shown greater benefits than the use of the same drugs for recurrent disease in normal hosts (Nilsen et al., 1982; Whitley et al., 1984). This improved efficacy may be because the relative absence of natural host antiviral defenses in these conditions and the resultant prolonged period of virus replication creates a broader target for chemotherapy. Topical antiviral treatment has been effective in immunocompromised patients with mucocutaneous HSV infection and in primary genital herpes (Corey et al., 1982; Whitley et al., 1984). However, systemic antiviral drug therapy is generally preferred under these circumstances to treat cervical or intraoral lesions and/or to cover the risk of disseminated HSV infection.

Recurrent labial and perioral HSV type 1 infection in normal hosts, the common fever blister or cold sore, is the most frequent cutaneous virus infection encountered. A topical treatment for this disease is both feasible and desirable: the lips are accessible for frequent topical drug application, the lesions are easily seen, and extracutaneous complications are uncommon. The topical route is an important and established mode of drug administration for herpes keratitis, but because of the numerous unique features of ocular chemotherapy, the reader is referred elsewhere (Liesegang, 1988).

The present review will focus specifically on topical treatments of recurrent HSV infections occurring in the cornified epithelium of immunocompetent hosts. Unlike infections of mucous membranes, topical cutaneous therapy is complicated by interposition of a potent anatomic barrier, the stratum corneum, between the site of drug application and the infection. Recurrent HSV infections in this category include genital herpes in men; selected cases of genital herpes in women, such as on the labia majora, perineum or buttocks; herpetic whitlow; and herpes labialis. The experience of the authors with herpes labialis will be used to illustrate the pathophysiologic and therapeutic issues which will be discussed.

The natural history of recurrent herpes simplex labialis

The treatment of recurrent HSV infections in immunocompetent patients is a formidable task because the developing infection is subject to the full impact of a healthy secondary immune response upon the reappearance of enveloped

virions into non-immunopriviledged tissue, such as from nerve axons into the skin, or the appearance of viral proteins on host cell surfaces. One can imagine a 'race' between viral replication, spread of infection to adjacent cells and viral-mediated lysis of keratinocytes; and immune factors acting to curtail the infection, including the neutralizing activity of antibody and complement, lysis of infected keratinocytes by lymphocytes and suppression of viral replication by lymphocyte-derived interferon (Rawls, 1985). By inference from clinical observations, the results of this 'competition' vary enormously. Herpes labialis ranges in severity from prodromal symptoms on the lips without lesion formation to papular lesions that resolve in 2–3 days to 'classical' herpes lesions with vesicle, ulcer and crust formation requiring 5–15 days for resolution (Spruance et al., 1990). It is unclear whether the variable outcomes in this disease are due to variable quantities of virus delivered to the skin, a variable intensity of the immune response, or a combination of both. To the extent that viral replication dominates temporarily over the immune response, antiviral chemotherapy has an opportunity to favorably influence the course of the disease.

The clinical course of herpes labialis may be conceptually divided into two phases, lesion development and lesion resolution, where the point of division is attainment of maximum lesion severity. Maximum lesion severity is defined as the largest number and size of lesions, evolution to the vesicle or ulcer lesion stage, and the maximum degree of pain. Lesion development occurs rapidly and maximum severity is reached, on the average, 8 h after the onset of the papular stage, likely through a process of simultaneous multifocal inoculation of the epidermis from the branching termini of infected sensory neurons (Spruance and Wenerstrom, 1984d). Antiviral drug treatment usually does not affect lesion development (Spruance et al., 1990).

Most of the clinical course of herpes labialis represents the phase of lesion resolution. To the extent that a defect in the epidermis has been produced, the phase of lesion resolution is analogous to healing of a small traumatic wound, which takes 7–10 days to resolve. While antivirals would be of no benefit to the healing of traumatically induced wounds, wound healing in herpes labialis is complicated by viral infection of keratinocytes and an inflammatory process proportional to the quantity of virus produced (Spruance et al., 1982a), factors which likely impair the process of re-epithelization. By reducing viral replication, antiviral therapy could theoretically accelerate healing.

Fig. 1 summarizes an hypothesis of the virologic, immunologic, and clinical events in herpes labialis with and without antiviral therapy. In this model, replication of virus in the epidermis, viral histopathology and a local immune response antedate awareness that a lesion is in progress. For this reason and because subsequent clinical events occur rapidly, development of a lesion to clinical maturity will likely not be altered by the early administration of antiviral drugs. However, if the drug effects a decrease in viral replication, this may manifest at a later time point during the phase of lesion resolution. The potential extent of this benefit is indicated by the difference in area under the curve for the course of treated and untreated lesions.

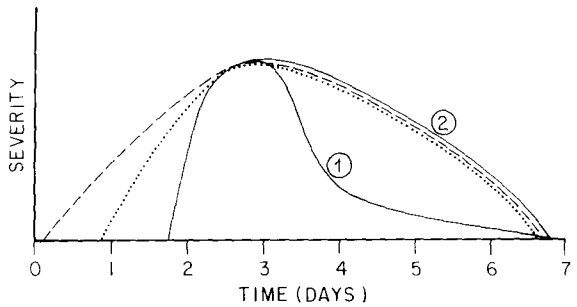

Fig. 1. Schematic representation of the possible course of events in the development of recurrent herpes simplex labialis. – – –, histopathology; ....., local cellular immune response; ___, clinical disease. Course of treated lesion (1) or untreated (2). (Reprinted with permission from Spruance, 1988a.)

Limitations of current antiviral substances

The antiviral activity of an ideal drug might have the following features: (1) an immediate and complete cessation of viral replication to curtail the spread of infection to new cells; (2) restoration or 'cure' of cells already infected with the virus at the start of treatment; and (3) inactivation of free virions. No single agent meets all these criteria. Most anti-herpesvirus therapy has been performed with nucleoside analogues such as ACV, the triphosphate form of which inhibits viral DNA polymerase and is incorporated into viral DNA (Elion et al., 1977). To what extent does ACV, the most commonly used antiviral substance, meet some of the criteria of an ideal drug? It does not inactivate free virus. Can ACV prevent the death of a cell already infected with HSV? Can it completely interdict HSV replication at achievable tissue concentrations?

The mechanism of HSV replication has been extensively reviewed elsewhere (Roizman and Batterson, 1985). Briefly, following penetration of the viral genome into the nucleus of the host cell, α- (immediate early), β- (early), and γ- (late) -genes are sequentially transcribed. Neither the α- nor the β-genes require replication of viral DNA prior to expression. Beta-gene expression includes the synthesis of the viral thymidine kinase (TK). At this point, ACV is preferentially phosphorylated by the viral TK leading to formation of its active derivative, ACV triphosphate. TK reaches its peak activity 6–8 h after infection (Klemperer et al., 1967). The γ-genes, which usually require viral DNA replication for expression, include sequences for the structural proteins of the virus, and some of these proteins are inserted into the host cell plasma membrane. Progeny virions are released 6–8 h after infection, and death of the infected cell follows shut off of host macromolecular metabolism and virus-induced alterations in cellular membranes (Roizman and Batterson, 1985). Virus-induced shut off of cell metabolism occurs in two stages: the first stage involves structural proteins of the virus and does not require de novo protein synthesis; the second stage requires synthesis of proteins and coincides with the onset of synthesis of β-proteins.

The interaction of HSV, host cell and ACV has been examined in vitro from sev-

eral aspects; experimental variables that influence the outcome include multiplicity of infection (MOI, PFU/cell), the timing of drug administration in relationship to the viral replication cycle, drug concentration, the permissiveness of the cell line, and virus strain. Harmenberg and Wahren (Harmenberg and Wahren, 1982) studied the influence of varying conditions on the activity of ACV against HSV in human fetal lung fibroblasts and green monkey kidney cells. They found that at low MOI (10^{-3} – 10^{-4}), high concentration ACV added at any time up to 7 h after infection caused significant inhibition of HSV-1 plaque formation in both cell types; but addition of ACV at later time points was increasingly less effective, and drug added at 48 hours post-infection had no effect. The data suggest that failure to completely suppress viral replication in the cells first infected resulted in progeny virions spreading to adjacent cells despite the later presence of the antiviral substance.

To study the effect of ACV on viral protein synthesis, Furman and McGuirt (1988) used a high MOI (10–20) HSV-1 infection in the presence of high concentration (100 μM) ACV. Immediate-early and early polypeptide synthesis were unaffected and late polypeptide and glycoprotein synthesis were reduced by 40–95% but not completely suppressed 16–24 h post-infection. Brice et al. (1988) infected human and rabbit keratinocytes with HSV-1 at MOI of 1–5 in the presence of 200 μM ACV and measured cell surface HSV antigen expression. They showed that although ACV reduced the proportion of cells with cytopathic effect at 24 h, the proportion of cells developing surface antigens was similar in ACV-treated and control cells: 95–99% of ACV-treated cells were antigen-positive 24 h after infection, in effect making them targets for lymphocyte-mediated cytolysis.

In mink lung cells with low MOI HSV-1 infections, delay or prevention of cytopathic effect by ACV is dose-dependent; but at an MOI of 0.5 or greater, infection eventually leads to complete loss of the monolayer regardless of the drug concentration (McKeough and Spruance, manuscript in preparation). In cultures of neuronal cells, the establishment of HSV latency is an alternative to cell lysis. ACV facilitates cell survival with latent virus up to a certain point but, with high MOI, the result is lysis (Wilcox and Johnson, 1988).

In summary, infection with HSV-1 at MOIs high enough (10–20) to insure infection of each cell, protein expression or cytopathic effect may be delayed by drug action, but the cell cannot be 'cured' by concurrent administration of ACV, even at high doses.

If 'cure' cannot be achieved with ACV, then maximum suppression of viral replication is desirable in order that additional cells not become infected. Antiviral drug potency is usually expressed as the amount of drug that inhibits a measure of viral replication in vitro by 50% (ID_{50}). While ID_{50} is a technically practical and useful measurement, an 'ID_{99}' may be more relevant to rapid suppression of viral spread in vivo. Barry et al. (1986) have noted the wide variation in ratios between ID_{50} and ID_{99} values in multiple HSV isolates and emphasized the fact that the mean ID_{99} for 20 isolates (4.21 μg/ml) was substantially higher than achievable levels following oral ACV (600 mg every 4 h giving a peak steady-state plasma concentration of 1.3 μg/ml) (deMiranda et al., 1983).

We do not know the 'MOI' of HSV-1 for basal keratinocytes during reactivation

in vivo, but in view of the large numbers of virions produced by permissive cells in vitro, it is reasonable to presume that closely neighboring cells in the epidermis may be exposed to a high number of progeny virions. The inverse relationship between ACV activity and MOI, the possibility of a high MOI virus-cell relationship in vivo, and the need to maximally suppress virus replication by achieving an 'ID_{99}' drug concentration rather than an ID_{50} argue that high tissue concentrations of ACV are needed to maximize its clinical effectiveness. In conclusion, insufficient delivery of ACV to herpes labialis patients with the current oral and topical drug formulations (deMiranda et al., 1983; Freeman et al., 1986a) should be considered one of the possible explanations for the limited effectiveness of ACV demonstrated to date against this disease (Table 1). Clinical dose-response curves should be part of topical drug development to identify the full potential of new compounds.

Breaching the stratum corneum: the role of penetration enhancers in topical antiviral therapy

For an antiviral drug to affect the course of recurrent cutaneous HSV infection in the immunocompetent patient, the drug must reach high virus-inhibitory levels in the cells targeted by the infection, the basal epidermal cell layers, very early in lesion development. At this time, there may be only prodromal symptoms or focal erythema. Therefore, topically applied therapy must penetrate essentially normal, intact skin. While the drug may more easily penetrate damaged skin as the lesion progresses into the vesicular and ulcer stages, it is too late at this point to expect much benefit from antiviral treatment.

The rate-limiting barrier to drug delivery through the skin is the stratum corneum, the outermost horny layer of the skin and the vermillion of the lips (Dimond et al., 1976). It functions primarily to prevent water loss and absorption of foreign materials and is ideally suited as a barrier with a rich lipid composition, particularly unesterified sterols, ceramides, and fatty acids (Bisset, 1987).

The stratum corneum is made up of alternating hydrophobic and hydrophilic layers consisting of flattened, non-living keratinocytes. It is continually renewed by maturing cells from the basal epithelial cell layer. As cells move up through the spinous and granular layers, they progressively flatten and keratinize until nuclei are absent and intracellular organelles disappear in the granular layer. These epidermal layers are rich in lytic enzymes necessary for cellular differentiation and transformation, and these enzymes may play a role in drug metabolism (see below). The flattened stratum corneum cells do not divide or grow, but enzymatic activity persists even in this layer to control desquamation (Bisset, 1987).

Topical therapy may be considered either 'active' or 'passive'. 'Passive' topical therapy incorporates no component altering skin permeability and includes the application of drugs in the majority of ointment, cream, and gel vehicles. 'Active' topical therapy implies penetration enhancement and may be achieved through mechanical, electrical or chemical methods. The mechanical method has been exemplified by Juel-Jensen and MacCallum's use of an air gun to deliver 0.1% IDU into

herpes labialis lesions (Juel-Jensen et al., 1965). While lesion duration was abbreviated, this method of assuring drug delivery has obvious drawbacks for clinical use. Electrical current to bear drug through the stratum corneum by iontophoresis has also been studied clinically (Gangarosa et al., 1986), with inconclusive results. The most widely studied and clinically feasible 'active' strategy has been 'chemophoresis' or the use of skin penetration enhancers (Cooper and Berner, 1987; Knepp et al., 1987).

An ideal penetration enhancer would act immediately and reversibly with a predictable duration of effect. The agent would not alter the thermodynamic activity (relative solubility) of the drug it is intended to deliver. It would also be specific and have no pharmacologic effects of its own. For practical use it would be nonirritating, physically and chemically stable and without odor or taste (Knepp et al., 1987). No currently available penetration enhancer satisfies all these requirements, but improved penetration enhancers will become available with continuing work in this field.

In general, penetration enhancers abrogate the barrier function of the skin by interacting with one or both of the two structural components of the stratum corneum: the hydrated, keratinized protein layers; or the lipid-rich 'grout' around and between the protein layers. One model considers three parallel pathways available for drug movement across the stratum corneum barrier: (1) a continuous polar or aqueous pathway composed of proteins; (2) a continuous nonpolar pathway composed of lipids; and (3) a pathway through a mixture of polar-nonpolar multilaminate lipids and proteins (Berner and Cooper, 1987). Increasing the rate of movement through the polar pathway may be achieved through solvent swelling or protein conformational change. The key to the nonpolar pathway may be increasing fluidity of the lipids.

Penetration enhancers may be loosely grouped into surfactants, solvents, and binary systems (Cooper and Berner, 1987). Surfactants appear to enhance the polar pathway of drug movement and include, for example, soaps and detergents. The usefulness of surfactants is limited by skin irritation, which tends to correlate directly with penetration enhancement. Solvents which are penetration enhancers include dimethyl sulfoxide (DMSO) and propylene glycol. Solvent effects on drug penetration are likely due to swelling of the polar pathway and/or fluidization of lipids. Binary systems, for example the combination of a lipid component with an unsaturated hydrocarbon chain and a polar solvent such as propylene glycol, may open up the third heterogeneous pathway in the stratum corneum. Laurocapram (Azone, 1-dodecylazacycloheptan-2-one), an extremely potent penetration enhancer, may be considered among the binary systems, although it's mechanism of action is incompletely understood (Vaidyanathan et al., 1987).

DMSO, probably the most studied penetration enhancer, is an excellent solvent, miscible with water and fairly easily incorporated into formulations. It is a strong hydrogen-bonding acceptor, as are other penetration enhancers such as Azone, Deet (*N,N*-diethyl-*m*-toluamide) and pyrrolidones (Sugibayashi et al., 1988). DMSO may act by eluting solvent-soluble components from the stratum corneum (Kurihara-Bergstrom et al., 1986), protein denaturation

TABLE 1

Selected trials of topical antiviral chemotherapy for herpes labialis

Reference	Drug & dosing	Therapy initiation	# Episodes/ # Patients	Findings	Investigators' conclusions (in quotes) and reviewers comments
MacCallum and Juel-Jensen, 1966	5% IDU in DMSO, t.i.d. for 3 days	Clinic, within 24 h	21/16	Trends to shorter time to crust and complete healing	Possible therapeutic value[a,b]
Spruance et al., 1979	10% araAMP cream, q.i.d. for 5 days	Clinic, 90% within 24 h of onset	233/233	No difference in clinical or virologic course	'Ineffective'
Rowe et al., 1979	3% araA gel, 6×/d for 7 days	Patient, as soon as possible	175/70	Faster lesion development, smaller lesions	'Not particularly effective'[b]
Spruance et al., 1982b	5% ACV ointment, 5×/d for 5 days	Clinic, within 25 h	208/208	Antiviral effect with early treatment[c]	'No significant clinical benefit'
Fiddian et al., 1983b	5% ACV cream, 5×/d for 5 days	Patient, within 24 h	74/49	Higher % 'aborted' lesions[c]; improved healing time[c]	'Effective'[a,b]
Fiddian and Ivanyi, 1983a	5% ACV ointment, 5×/d for 5 days	Patient, as soon as possible	31/13	Higher % 'aborted' lesions; shorter times to ulcer/crust and complete healing	'Therapeutic effect'[a,b]
Van Vloten et al., 1983	5% ACV cream, 5×/d for 5 days	Patient, within 12 h	60/30	Reduced vesicle duration,[c] times to crust and complete healing	'Reduced healing time'[a,b,d]
Spruance et al., 1984a	10% ACV ointment, 8×/d for 5 days	Patient, prodrome or erythema stage	69/69	No differences	'Ineffective'
Shaw et al., 1985	5% ACV cream, 5×/d for 5 days	Patient, within 24 h	72/45	No differences	'No significant therapeutic advantage'[b]; 1/3 of patients had vesicles when treatment was started.

Reference	Drug & dosing	Therapy initiation	# Episodes/ # Patients	Findings	Investigators' conclusions (in quotes) and reviewers comments
Lawee et al., 1988	3% PFA cream, q2h d1, q4h d2–5	Patient, as soon as possible	143/143	Reduced duration of viral shedding[c], more aborted lesions[c], and trend to less pain if treated before vesicle.	'No clinically important benefit'; strong suggestion that medication had efficacy if given very early.
Raborn et al., 1989a	5% ACV ointment 8×/d for 5 days	Patient, within 1 h of onset	60/60	No differences	'Not more effective than placebo'; similar findings in clinic-initiated study with same formulation
Raborn et al., 1989b	5% ACV cream, 5×/d for 5 days	Patient, within 1 h of onset	102/51	Trends to faster complete healing and smaller lesions	Possible benefit[a]
Spruance et al., 1990	15% IDU in DMSO, 6×/d for 4 days	Patient, within 2 h	301/301	Reduced pain duration[c] and time to loss of crust[c]	'Effective'

[a]Study too small to support firm conclusions regarding efficacy.
[b]Study includes re-treated patients as individual cases.
[c]Statistically significant at $P \leq 0.05$, test two-sided or not mentioned, assumptions valid for this variable.
[d]Data presentation incomplete.
[e]Statistical tests one-sided only.

and osmotic structural disruption from solvent cross-flows. The effect on the stratum corneum barrier is virtually immediate, an advantage for topical antiviral therapy. Relatively high (greater than 60%) concentrations of DMSO are required to significantly enhance penetration (Kurihara-Bergstrom et al., 1986).

What evidence is there that promoting drug delivery by enhancing skin penetration is important in topical herpes therapy? We have performed in vitro skin penetration studies with multiple active antiviral compounds and correlated the findings with the in vivo results in the dorsal cutaneous guinea pig model of HSV-1 infection (Freeman et al., 1985; Spruance et al., 1984b; Spruance et al., 1984c; Spruance et al., 1985). For each drug, formulation in a topical vehicle that enhanced skin penetration correlated with better efficacy in the guinea pig model, and in vivo efficacy was highly correlated with an expression combining the virus-inhibitory potency of each drug with the in vitro skin penetration results (Fig. 2) (Freeman and Spruance, 1986b).

In addition to its role as a barrier, the metabolic and enzymatic activities of the epidermis may also be important to drug transport and activity. Lipid soluble prodrugs, better able to penetrate the stratum corneum, may be hydrolyzed to active drug in the epidermis (Bisset, 1987). Conversely, hydrolysis in the epidermis may inactivate some agents. Enzyme activities may be altered when skin permeability is changed: the activity of HMG CoA reductase, the rate-limiting enzyme of cholesterol biosynthesis, is increased after barrier disruption in hairless mice (Proksch et al., 1989). Relative thymidine content of the epidermis may be important for the antiviral activity of nucleoside analogues which are competitive substrates with thymidine for the same enzymes (Ericson et al., 1985). In addition, pyrimidine nucleoside phosphorylases present in skin actively catabolize thymi-

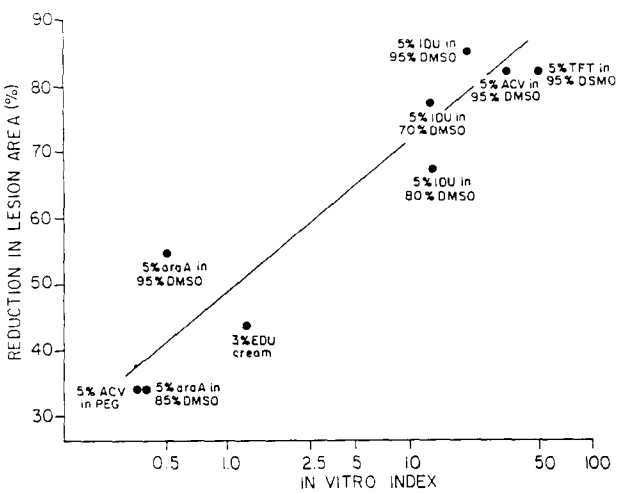

Fig. 2. Correlation of percent reduction in lesion area with the in vitro antiviral index in the dorsal cutaneous guinea pig model of HSV-1 infection for nine topical antiviral formulations. In vitro index = drug flux through skin/ID_{50}. Skin penetration studies performed at 37°C. (Reprinted with permission from Freeman and Spruance, 1986b.)

dine and some of the nucleoside analogues (Schwartz et al., 1988). The clinical significance of these local enzymatic activities for topical antiviral therapy is at present unknown.

Clinical trials with topical antiviral agents for herpes simplex labialis

Numerous clinical trials of topically applied antivirals drugs for the treatment of recurrent herpes labialis have been undertaken since the discovery of the antiherpesvirus activity of IDU in the early 1960's. However, demonstration of antiviral drug efficacy in normal hosts has been frustrating and elusive.

Experience has taught that a well-designed clinical treatment trial in herpes labialis must address several critical issues: the wide natural variability of the clinical episode requiring large numbers of study participants (200–300) to detect significant changes with drug intervention; the rapid evolution of symptoms and lesions in immunocompetent hosts and the importance of timely intervention (for example, study protocols with patient-initiated therapy); the marked placebo effect that mandates a double-blind, placebo-controlled study design; and the importance of adequate drug delivery to the site of infection, whether by the topical or oral routes of administration.

Table 1 reviews selected clinical trials of topical antiherpesvirus chemotherapy for the treatment of recurrent herpes labialis in immunocompetent hosts. All of the studies reviewed were double-blind, randomized, and placebo-controlled and evaluated the efficacy of known metabolic inhibitors of HSV-1. Assessment of the response to therapy was clinic-based and was as quantitative as possible. No protocol called for treatment to continue through the night. Despite the common elements in these studies, comparison of results is difficult because of variability in the approach to data analysis. Several trials have the relative design flaw of re-enrollment of the same individual for treatment of a second or third episode, and inclusion of these multiple episodes as independent events in the analysis. A more complete listing of herpes labialis therapeutic trials can be found elsewhere (Overall, 1984).

An early trial of topical 5% IDU in DMSO by MacCallum and Juel-Jensen (1966) showed a strong trend toward drug effect, but the number of patients studied was too small for the findings to reach statistical significance. This IDU formulation was developed for commercial purposes in Europe and is currently marketed for herpes labialis treatment. In 1979, adenine arabinoside (araA) as a 3% topical gel was studied in 70 patients (Rowe et al., 1979), and adenine arabinoside 5′-monophosphate (araAMP) as a 10% cream was evaluated in 233 patients (Spruance et al., 1979). No significant beneficial effects were seen in either study. Failure of treatment was attributed to poor skin penetration, in part because iontophoresis of araAMP in a mouse model of cutaneous HSV infection showed good activity (Park et al., 1978). Shortly thereafter, 5% ACV ointment was studied in 208 patients (Spruance et al., 1982b); antiviral activity was demonstrated, but again no clinical improvement could be documented among those who received the drug. Lack of

efficacy was attributed to poor skin penetration and late initiation of therapy.

Following these large studies and their disappointing results, study protocols generally incorporated patient-initiated treatment in an attempt to catch the early, brief window of opportunity when inhibition of viral replication might be expected to influence the subsequent lesion course. However, no effects on the course of herpes labialis were seen despite patient-initiated treatment with 10% ACV ointment (Spruance et al., 1984a). Other smaller trials of 5% ACV ointment with patient-initiated treatment reported some favorable trends but likewise unconvincing efficacy (Fiddian and Ivanyi, 1983a; Raborn et al., 1989a).

Acyclovir has also been formulated as a topical 5% aqueous cream, containing propylene glycol, a penetration enhancer, and with none of the negative pharmacokinetic properties that plague the ointment (polyethylene glycol) vehicle (Sheth et al., 1986). Evidence for better skin penetration from this formulation was seen in vitro (Spruance et al., 1986), and improved results over ACV ointment were found in animal studies (Collins and Oliver, 1982; Spruance et al., 1986). Four relatively small (30–49 patients) trials were undertaken using early, patient-initiated treatment with 5% ACV cream. Fiddian et al. (1983b) noted a reduction in healing time and significantly more ACV patients reporting aborted lesions. In a second group of 30 patients, both healing time and duration of the vesicle stage were reduced (Van Vloten et al., 1983). However, no statistically significant clinical benefit attributable to ACV cream was noted by Shaw et al. (1985) or by Raborn et al. (1989b). The use of another antiviral agent, foscarnet (PFA), in a topical aqueous cream formulation likewise led to promising but marginal results (Lawee et al., 1988).

While the skin penetration enhancer, propylene glycol, has been evaluated clinically in formulation with ACV and foscarnet, the alteration in the stratum corneum barrier function produced by this agent is minimal compared to what can be accomplished with DMSO (Freeman et al., 1986a). Recently, we treated 301 patients with recurrent herpes labialis with 15% IDU in 80% DMSO or a vehicle control solution in a large patient-initiated trial (Spruance et al., 1990). The mean duration of pain was reduced by 1.3 days (P=0.01) and the mean healing time (to loss of crust) by 1.7 days (P=0.004). Analysis of subpopulations revealed that the major benefit of the treatment occurred in the patients who had 'classical' herpes lesions (vesicle to ulcer/crust formation) and who began treatment in the prodromal or erythema lesion stages. Among these patients, the mean duration of pain was reduced by 2.6 days (P=0.03) and the mean healing time (to loss of crust) by 3.3 days (P<0.001). These positive effects with IDU in DMSO are highly encouraging and should promote continued clinical investigation of topical treatments for herpes labialis.

Future prospects and research directions

Extensive effort has been devoted over the past 20 years toward a topical therapy for cutaneous herpes simplex virus infections. There has been a growing understanding of what constitutes an adequate clinical trial and an adequate topical

formulation. Clinical trial results in herpes labialis have progressed from totally negative, to an antiviral effect without clinical benefit, to marginal clinical effects, to a statistically significant acceleration of lesion resolution with 15% IDU in DMSO (Spruance et al., 1990). No study has convincingly prevented lesion development (increased the number of 'aborted' lesions); this can be accomplished by prophylactic peroral administration of ACV (Lemak et al., 1986; Spruance et al., 1988b) but may not be achievable when treatment is begun at the onset of an episode.

Many questions remain to be answered. Is drug application at the first clinical symptom or sign of a recurrence quick enough intervention to effect a major change in lesion outcome? How much of lesion development is mediated by the host immune response as opposed to virus-directed cytolysis? We know very little about antiviral drug concentrations in the epidermis: how best to measure them, how to achieve the highest concentrations (peroral vs topical routes), and whether sustained or high, intermittent dosing is preferable.

Measurement of drug gradient or distribution in skin following topical application could be made by adhesive tape stripping, by heat or biochemical separation of skin layers or by skin sectioning with a microtome. Very little of this technology has been used to study antiviral compounds. Sheth et al. (1987) used adhesive tape stripping to document a correlation between the quantity of IDU in guinea pig stratum corneum and the therapeutic efficacy of topical IDU against cutaneous HSV-1 infection. Assessment of drug concentrations by tape stripping has the potential for large-scale in vivo human application since the technique is simple and relatively non-invasive.

A new method of studying human skin in vivo could add considerable dimension to our knowledge of drug delivery and localization. Human skin can be grafted onto a 'sandwich' flap with an isolated, accessible blood supply on the backs of nude rats. Drugs applied to the human skin surface can then be accurately measured in the venous return from the flap (Krueger et al., 1985; Pershing and Krueger, 1989). Drug levels in living skin can also be approached 'in reverse' – drugs which migrate from the systemic circulation into and through the skin can be measured from cutaneous reservoirs (Peck et al., 1988). Future applications of this technology to drug development and correlations with clinical outcome will define its role in the preclinical evaluation of topical antiviral formulations.

Carefully designed clinical trials with attention to logical disease measurements, early drug application and adequate population size should continue to advance our understanding of topical antiviral chemotherapy. Future studies should include the use of more potent antiviral agents; efforts to achieve higher drug concentrations in the target cells; treatment limited to the first two days of the disease and continuous treatment through the night time hours. Small screening studies with experimental ultraviolet light-induced herpes labialis could facilitate the selection of effective drugs and formulations (Spruance, 1988c).

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