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Susceptibility to herpes labialis following multiple experimental exposures to ultraviolet radiation ☆

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Abstract

We studied susceptibility to herpes labialis by exposing 20 volunteers to experimental ultraviolet radiation (UVR) on three occasions at 3- to 4-month intervals. The number of patients who developed lesions after each session was 9/20 (45%), 9/20 (45%) and 14/20 (70%). Herpes simplex virus (HSV) was isolated from 21/29 (72%) of lesions sampled. Three patients never developed a lesion, 13 developed lesions on one or two of the three occasions, and 4 patients had a lesion following all three sessions. Seven of 33 (21%) lesions were 'immediate' lesions (developed within 48 h) and the others developed 3–7 days after UVR exposure (delayed lesions). Development of lesions correlated with historical susceptibility to sun-induced herpes labialis, but not with age, sex, years with herpes labialis, frequency of herpes labialis from all causes, or concurrent serum levels of cortisol, dehydroepiandrosterone, estradiol, progesterone or α_1-antitrypsin. Among normally menstruating females, a significant association was identified between the development of herpes labialis and the luteal phase of the menstrual cycle (8 cases of herpes labialis/11 attempts, RR = 14, $P = 0.005$). The lack of correlation between episodes of natural herpes labialis and susceptibility to experimental UVR-induced disease suggests that these events are controlled differently. The results of serial attempts to induce experimental herpes in each

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patient was most commonly inconsistent, indicating that individual patient susceptibility to UVR varies over time. While the explanation for this variation remains unclear, stages of the menstrual cycle may be important among women.

Keywords: Herpes simplex labialis; Herpes simplex virus; Immunity; Ultraviolet radiation; Menstrual cycle; Luteal phase

1. Introduction

A number of 'trigger' factors for recurrent herpes labialis have been reported by patients, including emotional stress, illness, exposure to sun, trauma, fatigue, menses, chapped lips, and season of the year. Fever, trigeminal nerve surgery, dental work and epidural anesthesia have also been reported by health care professionals to precipitate recurrences (Spruance, 1992). The mechanisms that lead to reactivation of herpes simplex virus (HSV) in these various circumstances are unclear.

It has been tempting to speculate that changes in circulating hormone levels mediate some recurrences. Pregnancy is associated with an increased frequency of recurrent HSV infections, depression of cell-mediated immunity and high levels of circulating estrogen and progesterone (Weinberg, 1984; Brown et al., 1985). Mice are more susceptible to inoculation with HSV-2 during pregnancy, during the diestrous phase of the murine menstrual cycle and when treated subcutaneously with progesterone (Baker and Plotkin, 1978; Teepe et al., 1990). Mice with experimentally enhanced levels of dehydroepiandrosterone (DHEA) develop a more efficient Th1-like immune response, whereas administration of glucocorticoids promotes Th2-like antigen-specific cytokine production (Daynes and Araneo, 1992; Kay, 1994).

There is recent evidence to suggest that neutrophils may play a role in localized cell-mediated immune responses (Lee et al., 1988), the type of host resistance that may be responsible for controlling recurrences of HSV infections (Schmidt and Mawle, 1991). The degradation products of neutrophil proteases are chemotactic for mononuclear cells, and inhibition of protease activity with α_1-antitrypsin depressed experimental delayed-type hypersensitivity reactions (Lee et al., 1988). Accordingly, we have speculated that variations in the concentration of a serum protease inhibitor such as α_1-antitrypsin might explain variations in susceptibility to recurrences of herpes labialis.

The experimental model of ultraviolet radiation (UVR)-induced herpes labialis provides an opportunity to investigate the factors associated with HSV reactivation because the investigator has control of the time when the reactivation may occur (Spruance et al., 1991). In the present study, we challenged individuals with UVR 3 times at 3- to 4-month intervals to determine the consistency of susceptibility and to correlate individual variations in host factors with the occurrence of experimental lesions.

2. Materials and methods

2.1. Study design

Twenty-eight patients were enrolled in a longitudinal study of the natural history of experimental UVR-induced herpes labialis. A medical history was taken on each patient

and HSV-1 serologic status was confirmed by serum neutralizing antibody assay and Western blot. Patients were induced with UVR three times at 3- to 4-month intervals and followed for the development of herpes labialis as described below. At the beginning of each session, we measured plasma levels of the steroid hormones cortisol, DHEA, DHEA-sulfate (DHEAS), estradiol, progesterone and α_1-antitrypsin to see if these substances conferred susceptibility or resistance to the development of experimental herpes labialis. Irradiation sessions were delayed, as necessary, such that there were no spontaneous episodes of herpes labialis within 30 days of each UVR exposure. We also correlated the development of experimentally induced lesions at the first irradiation session with measures of humoral and cellular immunity and have reported these findings elsewhere in this journal (Spruance et al., 1995).

2.2. Patient population

All subjects experienced frequent recurrent herpes labialis ($\geqslant 3$ episodes/year) and had a history of reactivations following sun exposure. The patients were in good health without major underlying medical conditions and were not taking any immunosuppressive treatments or chronic prophylactic antiviral therapy. All patients signed an Institutional Review Board-approved document of informed consent after the nature of the procedure was fully explained.

2.3. Exposure of the lips to UVR

The source of UVR were two fluorescent tubes (FS20, National Biological Corp., Twinsburg, OH) which emitted 17 mJ/cm^2/min of energy under the circumstances of these experiments. The patient positioned his/her lips in the opening of an opaque shield and was irradiated with 4 minimal erythema doses. The full details of this procedure are available elsewhere (Spruance et al., 1991, 1995).

2.4. Evaluation of lesions

Patients were monitored for 7 days after irradiation for evidence of herpes labialis. The exact time of lesion onset was obtained historically by patient interview and was defined as the patient's first awareness of a papule or induration. Lesions developing within the first 48 h after irradiation were termed 'immediate' lesions, and within 3–7 days, 'delayed' lesions. 'Site-specific' lesions were those that developed within the area of UVR exposure or within 1 cm of the exposure zone. The other lesions were called 'non-contiguous'. An episode was said to consist of more than one lesion when sores were separated from one another by more than 1 cm. An 'aborted' lesion did not progress beyond the papule stage while those that evolved to the vesicular, ulcerative and/or hard-crust stages were considered 'classical' lesions (Spruance et al., 1990).

Clinical assessment of lesion severity was made by observation of lesion stage, size, and pain. Lesion specimens for virus isolation were taken during the first 3 lesion days by breaking vesicles and absorbing vesicle fluid into a cotton swab and swabbing the base of ulcers (Spruance et al., 1990).

2.5. Virus isolation

Lesion swab specimens in virus transport medium were plated onto monolayers of mink lung cells. The presence of HSV in the specimen was determined by the appearance of characteristic cytopathic effects (Salmon et al., 1984).

2.6. Serology

Complement-independent neutralizing antibody titers were determined from serial dilutions of heat-inactivated serum by standard procedures (Rawls et al., 1970; Spruance et al., 1995). Assays for HSV-1- and HSV-2-specific antibodies were performed by Western blot by Dr. Rhoda Ashley, Children's Orthopedic Hospital, Seattle, WA (Ashley et al., 1991).

2.7. Assay of plasma steroids

Blood was drawn at the time of irradiation, between 08.00 and 10.00 h, for determination of plasma cortisol, DHEA, DHEAS, progesterone, and estradiol levels. Assays were performed with commercially available kits by radioimmunoassay procedures according to manufacturers' instructions (Coat-A-Count, Diagnostic Products Corp., Los Angeles, CA; DHEA Kit, Diagnostic Systems, Webster, TX).

Among normally menstruating female patients ($n = 13$), the menstrual cycle phase at the time of each irradiation was determined using plasma progesterone and estradiol concentrations and, when required, the date since the last menstrual period (see Table 1). In order for patients to be considered to be in the midcycle phase, 13–16 days must have elapsed since the last menstrual period in addition to the hormonal criteria given in Table 1. Patients taking oral contraceptive pills and postmenopausal patients were not scored into a particular menstrual phase.

2.8. Serum α_1-antitrypsin determinations

Assays were kindly performed by Edward J. Campbell, Salt Lake City Veteran's Administration Hospital (Silverman et al., 1988).

2.9. Statistical analysis

The data were screened for potentially significant differences using the Chi-square test for discontinuous data and Student's t-test for continuous variables. Because

Table 1

Menstrual Phase	Progesterone (ng/ml)	Estradiol (pg/ml)
Follicular	< 1.5	10–375
Midcycle	< 2.0	> 120
Luteal	> 2.5	50–260

Table 2
Characteristics of the study population

Patients exposed to UVR three times (n)	20
Age, median years, (range)	37 (22–67)
Gender (% women)	85
Race (% Caucasian)	100
Time with herpes labialis, median years (range)	22 (3–56)
Episodes of herpes labialis in the last 12 months, median # (range)	3.5 (2–14)
Frequency of herpes labialis after sun exposure	
Less than 50% of the times (% of patients)	35
More than 50% of the times (% of patients)	65
Skin type	
Type 2 (usually burn, seldom tan) (%)	35
Type 3 (sometimes burn, tan normally) (%)	65
Time since last natural episode of herpes labialis, median days (range)	62 (30–191)

patients underwent experimental irradiation up to 3 times each, data points were not truly independent events. Therefore, purported associations with a screening P-value less than or equal to 0.05 were analyzed using multiple logistic regression for correlated data (Liang and Zeger, 1986).

Other comparisons between groups were performed by the Chi-square test, ANOVA under the assumption of normal distribution of data, and by the non-parametric Kruskal–Wallis procedure. All P-values were two-tailed.

3. Results

3.1. Characteristics of the study patients

Of the 28 patients who enrolled in the study, 20 were fully compliant with the protocol and were exposed to experimental UVR on 3 occasions at intervals of 3–4 months. The other 8 persons missed one or more sessions because of conflicting personal activities or the development of a natural lesion near the time of their appointment. All subjects were confirmed to have HSV-1 serum antibodies by Western blot. Four individuals had antibodies to both HSV-1 and HSV-2. The characteristics of the patients are shown in Table 2. The majority were young, white women.

3.2. Development of herpes labialis following UVR exposure

Among the 20 patients who were irradiated 3 times, there was a total of 32 episodes of UVR-induced herpes labialis and 37 separate lesions. HSV was recovered from 21/29 (72%) of lesions sampled. The frequencies with which episodes were induced following the 3 radiation exposures were 9/20 (45%), 9/20 (45%) and 14/20 (70%). The difference in reactivation rates between the 3 UVR exposure sessions was not significant ($P = 0.19$). There was great variability among the individual patients in the

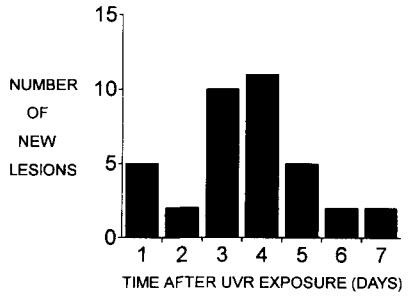

Fig. 1. Time course of the development of herpes labialis lesions in the 7 days following exposure to experimental UVR. Each lesion is counted once according to the day of onset. Lesion onset was defined as the day on which the papular stage was first observed. This figure shows the cumulative number of lesions experienced by 20 patients following 3 UVR exposure sessions. For the 60 times that patients were at risk, there were a total of 32 episodes of herpes labialis and 37 separate lesions.

number of times that UVR exposure effected a recurrence of herpes labialis, ranging from none (3 patients) to once (6 patients), twice (7 patients) and all 3 occasions (4 patients). Patients who had only one recurrence were most likely to develop the disease at the third UVR exposure session ($P = 0.02$).

The time of lesion development postirradiation was bimodal, confirming prior observations that there are both 'immediate' (0–48 h post-UVR) and 'delayed' (3–7 days post-UVR) types of lesions induced by UVR exposure. There were 7 immediate lesions and 30 delayed lesions (Fig. 1). There were 3 patients whose only reactivation was one immediate lesion; 3 patients had immediate lesions at one session and a delayed lesion on another occasion; and one patient had both and an immediate and a delayed lesion at the same irradiation session. Three immediate lesions occurred following the first irradiation session, none following the second session, and 4 following the third session. Virus was isolated at similar rates from immediate (3/4, 75%) and delayed lesions (17/25, 68%).

Most of the UVR-induced lesions were site-specific (29/37, 78%). Of the 8 non-contiguous lesions, 3 were the only lesion in that episode and 5 occurred in addition to a site-specific sore. All in the latter group of lesions began on a different day than the site-specific sore(s) in the episode. Two of the non-contiguous lesions began within 48 h of irradiation (immediate lesions) and 6 after 3–7 days (delayed lesions). Four non-contiguous lesions occurred following the first irradiation session, two after the second session, and two after the third session.

Of the 37 UVR-induced lesions, only one was 'aborted' and the remainder were 'classical' lesions (Spruance et al., 1990). The median lesion size was 135 mm^2 and the range was 20 to 792 mm^2. Lesion size and lesion duration were similar following the 3 irradiation sessions, and there was no significant difference in lesion size or duration among patients who developed lesions once, twice or following all 3 UVR exposures (data not shown).

The number of times that the patients developed herpes labialis following 3 experimental UVR challenges correlated with their self-described susceptibility to cold sores

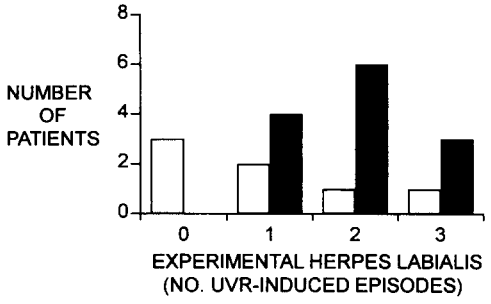

Fig. 2. The cumulative number of UVR-induced episodes of herpes labialis among 20 patients each of whom was irradiated 3 times. In addition, the development of experimental disease was correlated with historical susceptibility to cold sores from sun exposure: white columns, herpes after sun exposure less than 50% of the time; black columns, herpes after sun exposure more than 50% of the time. The difference in experimental lesion frequency between these two subgroups was statistically significant ($P = 0.05$).

from sun exposure, but not with their reported frequency of natural herpes labialis from all causes in the year prior to the study (Figs. 2 and 3). Patients with type 2 skin (usually burns, seldom tans) trended toward more experimental lesions than those with type 3 skin (sometimes burn, tan normally), but the results were not statistically significant (data not shown). The 4 patients with both HSV-1 and HSV-2 serum antibodies experienced only 3 experimental lesions in 12 attempts. However, 3 of these 4 individuals were self-described as low-risk for sun-induced recurrences which could explain their apparent reduced susceptibility. Development of experimental lesions did not correlate with age, sex, or number of years with herpes labialis.

3.3. Correlation of lesions with plasma hormone levels

An analysis of plasma hormone levels was conducted among 25 women, including some individuals who did not complete all of the three irradiation sessions. There were

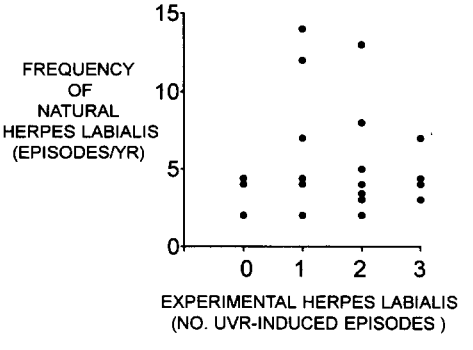

Fig. 3. Correlation between the cumulative number of experimental UVR-induced recurrences in this study and the reported number of episodes of naturally occurring herpes labialis in the preceding 12 months. There was no significant relationship ($r = -0.02$, $P = 0.63$).

Table 3
Mean levels of plasma hormones at the time of irradiation among 25 women with and without herpes labialis following UVR exposure

Mean plasma hormone	Experimental site-specific [a] herpes labialis		RR [b]	95% CI [c]	*P*-value
	Yes	No			
Cortisol (μg/dl)	19.1	17.6	1.02	0.97–1.07	0.51
DHEA (ng/ml)	2.6	3.5	0.80	0.63–1.02	0.07
DHEAS (μg/ml)	0.9	1.3	0.31	0.08–1.18	0.09
Estradiol (pg/ml)	147	106	1.00	0.99–1.00	0.27
Progesterone (ng/ml)	4.1	2.2	1.09	0.97–1.22	0.14

[a] Lesions developing within the area of irradiation.
[b] RR, relative risk, by multiple logistic regression for correlated data (Liang and Zeger, 1986).
[c] CI, confidence interval.

insufficient numbers of men in the study to analyze their values. Twelve of the women were postmenopausal, posthysterectomy, or taking oral contraceptive pills at the time of irradiation. Thirty herpes labialis episodes were experimentally induced in the 25 women from a total of 58 attempts (30/58, 52%). In twenty-eight of the recurrences, the lesions were site-specific.

Mean plasma DHEA and DHEAS levels tended to be lower and progesterone levels higher among the women who developed lesions compared to those who did not, but no statistically significant differences in plasma hormone levels by susceptibility to experimental lesions were found (Table 3). In addition, no significant differences in hormone levels were detected between women with a history of herpes labialis, HSV-seropositive history-negative subjects and HSV-seronegative controls (data not shown).

Thirteen women were available to study the association of herpes labialis with the normal menstrual cycle. There were 28 lip irradiations conducted among these 13 women which led to 15 recurrences, in 14 of which the lesions were UVR site-specific. The frequency of UVR-associated lesions, particularly site-specific lesions, was influenced by the stage of the menstrual cycle. The rates of site-specific lesion development during different menstrual stages were: follicular (3/11, 27%), midcycle (3/6, 50%), and luteal (8/11, 73%) (Table 4). The relative risk of developing herpes labialis for women in the luteal phase was 14 by multiple logistic regression (95% confidence interval 2.2–88, $P = 0.005$). Comparison of postmenopausal women, women using oral contraception and women menstruating normally revealed no significant differences (data not shown).

3.4. Correlation of experimental lesions with serum anti-protease levels

Among 28 patients who were available for the first irradiation session, UVR-induced herpes labialis developed among 5/8 (62%) patients with low or low–normal levels of serum α_1-antitrypsin and among 7/20 (35%) patients with normal serum enzyme

Table 4
Development of UVR-induced herpes labialis among 13 normally menstruating women according to the stage of the menstrual cycle

Menstrual phase	Experimental site-specific [a] herpes labialis			RR [b]	95% CI [c]	*P*-value
	N [d]	Yes	No			
Follicular	11	3	8	1.0	–	–
Midcycle	6	3	3	5.3	0.3–82	0.23
Luteal	11	8	3	14	2.2–88	0.005

[a] Lesions developing within the area of irradiation.
[b] RR, relative risk, by multiple logistic regression for correlated data (Liang and Zeger, 1986). Relative risk of developing site-specific herpes labialis is in comparison with patients in the 'follicular' group.
[c] CI, confidence interval.
[d] Number of irradiation events of patients in each particular menstrual phase.

levels. This difference was not statistically significant ($P = 0.36$). In addition, there was no significant difference in the frequency of low or low–normal enzyme levels among patients with a history of herpes labialis, HSV-seropositive history-negative subjects and HSV-seronegative controls (data not shown).

4. Discussion

We studied susceptibility to herpes labialis by exposing 20 volunteers to experimental UVR on 3 occasions at 3- to 4-month intervals. The number of patients with lesions at each session ranged from 45 to 70%, and 17/20 (85%) of the subjects were successfully induced at one or more of the sessions. Development of lesions correlated with historical susceptibility to sun-induced herpes labialis, but not with age, sex, years with herpes labialis, frequency of herpes labialis from all causes, or concurrent serum levels of cortisol, dehydroepiandrosterone, estradiol, progesterone or α_1-antitrypsin.

We identified that the development of herpes labialis following experimental UVR exposure was significantly more likely to occur among women in the luteal phase of the menstrual cycle compared with those in the follicular phase (8 cases/11 attempts vs 3/11, RR = 14, $P = 0.005$). A weak association between higher levels of progesterone in the blood at the time of irradiation and development of herpes labialis also was found, which, however, paralleled the much stronger association between the luteal phase of the menstrual period and susceptibility to experimental herpes labialis. While the exact reason for the increased susceptibility to women in the luteal phase of the menstrual cycle is not known, progesterone has been associated with a variety of effects on the immune system, including down-modulation of cell-mediated immune responses (Baker et al., 1980; Schuurs and Verheul, 1990).

The lack of correlation between episodes of natural herpes labialis and susceptibility to experimental UVR-induced disease in this study suggests that these events are

controlled differently. Studies of immune measures and susceptibility to UVR provide support for this observation. In additional investigations of the present study subjects reported elsewhere in this journal (Spruance et al., 1995), we compared serum HSV-neutralizing antibody, peripheral blood mononuclear cell interferon-γ and interleukin-2 production among patients with frequent natural herpes labialis, HSV-seropositive history-negative subjects, and HSV-seronegative controls and the same immunologic parameters among patients who could be induced to have a recurrence by UVR exposure and those who could not. UVR-induced lesions appeared to be facilitated by type 1 immunity (increased levels of interferon-γ and interleukin-2), which was opposite from the apparent role of these cytokines in resistance to natural episodes of disease.

The results of serial attempts to induce experimental herpes in each patient were most commonly inconsistent, indicating that individual patient susceptibility to UVR varies over time. One possibility, the 'skin trigger' hypothesis (Hill and Blyth, 1976), is that HSV is shed periodically into the skin and lesions develop only when the skin is susceptible to infection, such as might occur with UVR exposure (Otani and Mori, 1987). However, when we examined saliva for evidence of HSV immediately prior to experimental UVR exposure, on the presumption that asymptomatic shedding into the skin might be paralleled by shedding into the oral cavity (Spruance, 1984), we found no correlation with the subsequent appearance of UVR-induced lesions (Kriesel et al., 1994). Other studies have suggested that fluctuation in HSV-specific immune factors could be responsible (Cunningham and Merigan, 1983; Torseth and Merigan, 1986; Kuo and Lin, 1990). While the explanation for individual variation in susceptibility remains unclear, the present study provides evidence that stages of the menstrual cycle, possibly mediated by the effects of progesterone on immune function, may be an important contributing factor among women.

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