

## Treatment of Recurrent Herpes Simplex Labialis with Levamisole

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Because deficient immune responses may play a contributory role in recurrent herpes simplex labialis, an immunomodulating agent, levamisole, has been advocated for therapy. Forty-two patients with a high frequency of recurrent herpes simplex labialis were followed for a mean of 7.8 months (range 4 to 12) and treated for 3 days at the onset of each episode of herpes with one of three different doses of levamisole or placebo in a randomized, double-blind study. Statistical analysis revealed that as the dosage increased, so did the frequency of recurrences ( $P = 0.007$ ). Conversely, duration of the lesions and lesion pain decreased with increasing dosage ( $P = 0.05$  and  $0.03$ ). These results indicate that levamisole is not an appropriate drug for the management of recurrent herpes simplex labialis. The paradoxical response to an immunomodulator (increased frequency, decreased severity) provides evidence that altered host responses may contribute to the pathogenesis of the disease.

Because recurrent herpes simplex labialis (HSL) is more severe in immunocompromised subjects (6, 8) and because it has been hypothesized that a defect(s) in cell-mediated immunity may play a contributory role in otherwise normal subjects with recurrent HSL (7), investigators have felt that an immunomodulating agent might be beneficial in altering the course of the disease (3, 11). Numerous workers have demonstrated that levamisole affects the function of polymorphonuclear leukocytes, macrophages, and T-lymphocytes in humans and in animals (12). Initial evaluation of levamisole in herpes simplex virus infections consisted of uncontrolled studies in which 150 mg of the drug was administered orally on an intermittent basis, 2 or 3 consecutive days every week or every other week (3, 11). Reduced severity and frequency of recurrent HSL were claimed, although the lack of control groups and the known placebo effect on the course of this disease leave the significance of these investigations open to question (7). This report presents the results of a different protocol, in which 42 patients with recurrent HSL received either placebo or three different daily dosages of levamisole taken the first 3 days of each new episode of HSL. We were able to show that levamisole reduced lesion severity

but, unfortunately, also caused an increase in the frequency of recurrences.

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### MATERIALS AND METHODS

**Patient recruitment and criteria for inclusion in the study.** Patients were solicited by advertising in the community. Eligible candidates weighed 100 pounds (ca. 45.4 kg) or more, were not pregnant or planning pregnancy, had no underlying medical illnesses, were not taking any immunosuppressive medications, and had a history of recurrent labial or perioral fever blisters at a frequency of not less than one every 2 months. Human Studies Committee approval was obtained, and each participant signed a consent form. Each participant was evaluated initially at the time of an active lesion. A lesion specimen for isolation of herpes simplex virus was taken, and a Tzank smear was made by scraping the base of the lesion. A leukocyte count, a brief medical history, and the patient's recollection of the characteristics of his recurrent lesions were obtained. If the culture was positive and/or the Tzank smear showed multinucleated giant cells, the patient's history of recurrent HSL was considered validated and he was enrolled into the study. Patients were included in the study analysis if they took at least one course of medication and were followed for a minimum of 120 days.

**Study Design.** Subjects whose lesions were confirmed as recurrent HSL returned to the clinic to

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receive medication for the next episode of HSL. Patients were instructed to take the medication for three consecutive days, beginning with the first sign or symptom of the next recurrence. There were four randomized treatment groups: placebo and 50, 100, or 150 mg of levamisole per day. The placebo group was designed to be twice as large as the other groups. Placebo and active medication were contained in capsules so that the levamisole could not be detected by taste. On day 3 to 5 after lesion onset, the patient returned to the clinic and was asked to evaluate the overall discomfort of the present lesion and to describe any adverse reactions. Another peripheral leukocyte count was obtained, and the patient was given medication for the next episode. In addition, the patient kept a record at home of the number of days of lesion pain, the number of days that the lesion was present (until loss of crust), and whether the pain and duration of the episode were different, relative to pretreatment lesions. Pain was graded according to the following scale: (1) no pain; (2) mild; (3) moderate; and (4) severe (10). If patients failed to have recurrent episodes, they were telephoned every 2 months to document the absence of lesions.

**Virology.** Attempts to isolate herpes simplex virus were performed on primary rabbit kidney cells as previously described (2).

**Statistical methodology.** Analysis of the statistical significance between levamisole dose groups was performed using the Mantel-Haenszel procedure (4). This technique allowed for control of previous lesion frequency in the analysis of the frequency of lesions during the trial. The method of least squares was used to fit lines to the data displayed in Fig. 1. The correlation coefficient, *r*, was used to show the strength and direction of association, but was not used for formal statistical testing.

**RESULTS**

**Efficacy.** Sixty-three individuals met the clinical and laboratory criteria for admission to the study. Eleven of these never took the medication and are not considered further. Ten others remained in the study for less than 120 days. The remaining 42 persons are the source of data for analysis. The control and treatment groups were

similar with respect to age, sex, and previous experience with lesion pain and duration (Table 1).

The measurements of the course of HSL chosen for the study were: (i) the observed frequency of recurrent episodes; (ii) the overall lesion pain as judged by the patient on day 3 to 5 after lesion onset; and (iii) the lesion duration as marked on the patient's home record. The number of treated episodes per patient ranged from 1 to 16, with a mean of 4.9. Mean values for lesion duration and lesion pain were derived for each patient from each individual's several or more disease episodes. These means and the observed lesion frequency were then evaluated as a function of drug dosage. As shown in Fig. 1, there was a positive association between drug dosage and lesion recurrence rate (*P* = 0.007). In contrast, there was a negative correlation between drug dosage and lesion pain (*P* = 0.033) and between drug dosage and lesion duration (*P* = 0.051). In clinical terms, the group that received 150 mg of levamisole per day experienced a doubling of their recurrence rate in comparison with the placebo group, a mean reduction in pain severity equivalent to half the difference between mild and moderate, and a reduction in mean lesion duration of 2.3 days.

To substantiate the validity of the observation that treatment increased the lesion recurrence rate, prestudy lesion frequency in the placebo and treatment groups was further evaluated. A Mantel-Haenszel analysis indicated that there was a positive relation between treatment dosage and prestudy lesion frequency, but at a low level of statistical significance (*r* = 0.17, *P* = 0.28). Patients with different prestudy lesion recurrence rates were then analyzed separately, and the results were combined in a summary Mantel-Haenszel chi-square test, a procedure that eliminates bias by patient stratification (4). Even with this more conservative statistical

TABLE 1. Characteristics of the study population

Characteristic	Control group	Treatment groups
Number admitted to the study	26	37
Number (%) qualifying for the analysis	17 (65)	25 (68)
Analysis subgroup		
Average age (years)	28 ± 10.5 <sup>a</sup>	27 ± 8.4
Males (% of patients)	24	20
Past lesion frequency (% of patients)		
More than once a month	12	24
Every 1 to 2 months	88	76
Less than once every 2 months	0	0
Average pain score previously experienced	2.9 ± 0.6	2.7 ± 0.6
Average previous lesion duration (days)	9.6 ± 2.7	9.4 ± 3.0

<sup>a</sup> One standard deviation.

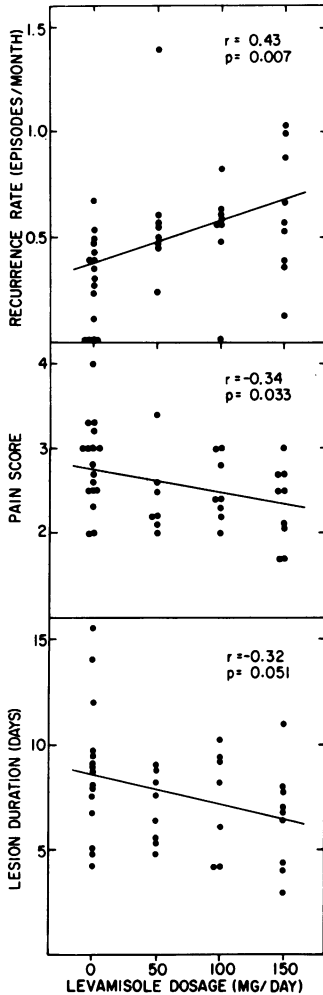


FIG. 1. The effect of different doses of levamisole on the rate of recurrence, pain, and lesion duration in patients with recurrent HSL.  $r$  is the correlation coefficient and  $P$  is the statistical probability, the latter determined by the Mantel-Haenszel procedure. Each dot represents one patient, and the value shown is the mean derived from several or more episodes of recurrent HSL. Because of missing data, the number of patients shown in some of the analyses may be less than the group total.

methodology, which will reduce the level of statistical significance even in the absence of any bias, significant positive correlation between treatment dosage and observed lesion frequency remained ( $P = 0.023$ ).

**Safety.** Levamisole toxicity could be evaluated in 52 patients who had at least one course of levamisole. The total number of 3-day courses of treatment was 220. Two patients were dropped from the study: one on the 150-mg/day dosage had intolerable gastrointestinal disturb-

ances, and another individual left the study upon hearing of reports of a relationship between levamisole and leukopenia. Five individuals had a leukocyte count below  $4,000/\text{mm}^3$  on one occasion, two of whom were in the placebo group and three in the treatment groups. Subsequent leukocyte counts in these individuals were normal. Statistical analysis of all leukocyte counts performed showed no significant correlation with dosage or number of treatments.

The incidence of adverse symptomatology was 9% in the placebo group (6 of 66 episodes) and 14% in the levamisole-treated groups (22 of 154 episodes). Nausea occurred in 4 of the 10 subjects treated with 150 mg of levamisole per day and in 1 of the 24 placebo patients. Disturbances of smell and taste (3 patients) and central nervous system symptoms such as headache and dizziness (5 patients) occurred only in the treated patients.

## DISCUSSION

In spite of considerable variability in response at each dose, the data in this study suggest that an immunomodulating drug, levamisole, can cause a dose-dependent decrease in the duration and pain of recurrent HSL and simultaneously increase the recurrence rate. In view of these unusual findings, it is of interest to review the available data on the relationship between lesion pain, duration, and frequency in untreated subjects. Rand et al., in a study involving immunosuppressed cardiac transplant patients (8), has shown that both the severity of lesions and the frequency of lesions are increased during the period of immunosuppression. In a study of 788 normal, untreated persons in a general practice in Wales, Grout and Barber also identified a positive correlation between frequency and severity (1). There is no evidence that frequency is inversely related to lesion severity in normal or immunosuppressed subjects, as noted in this study. These observations suggest that an unusual alteration in the host-parasite relationship may be induced by levamisole, and they lend credence to the hypothesis (7) that altered host cellular responses may play a role in the pathogenesis of the disease.

There have been two other double-blind, placebo-controlled studies evaluating levamisole in the treatment of HSL. Mehr and Albano (5) used the same protocol described in the present report, except that only the 100-mg/day dosage was employed. Lesion duration was no different between control and treated individuals. Insufficient data were available to comment on the effect of therapy on lesion frequency. The negative results in this report might be attributable

to the small size of the study (57 treated episodes versus 206 in our study). In contrast to the protocol of Mehr and Albano and ourselves, in which the drug is administered concomitantly with the onset of each episode of HSL, others have given levamisole on a regular schedule irrespective of the time of occurrence of the disease. Russell et al. (9) gave 99 subjects either placebo or 2.5 mg of levamisole per kg on two consecutive days each week for 6 months. Lesion duration was not different between the two groups during the treatment period, but lesion frequency was reduced 56% in the levamisole group (compared with prestudy lesion frequency) and only 20% in the placebo group, a statistically significant difference ( $P < 0.01$ ). These results sharply contrast with our own. The difference in results may be related to the total amount of levamisole administered, which was fivefold more on the average in the study of Russell et al. than in ours, or to the timing of "immunomodulation," which occurred only at the time of appearance of herpes antigen in our study.

The possibility that our unusual and unconfirmed results might be spurious needs to be considered. A great number of clinical trials are performed each year, and a few will report statistically significant but erroneous results, by chance alone. Second, randomization and blinding only reduce the likelihood of bias in a trial, and poor allocation of patients for some unknown factor(s) may be the real cause of the association found. In a small study such as ours, misplacement of only a few patients may be sufficient to influence the results. Finally, the occurrence of adverse symptomatology more frequently in the levamisole group may have induced greater psychological effects among the drug recipients.

In defense of our results, our data on recurrence rates are a simple record of the number of lesions over a period of time and are likely to be reasonably reliable. Most of the reported lesions were confirmed in the clinic, and the significance of the positive correlation between dose and recurrence rate was high ( $P = 0.007$ ). In addition, we carefully looked for sources of bias in our analysis and controlled for any known discrepancies in the allocation of patients. The mean lesion duration in our placebo group was high (8.8 days), yet this is not dissimilar from the value of 8.0 days until loss of crust that we have

reported for unmanipulated control patients with recurrent HSL (10).

This study indicates that levamisole should not be used in the management of recurrent HSL because it may increase lesion frequency while causing only a mild reduction in lesion severity. More investigation is needed to define the precise host defect(s), if any, that contribute to recurrent HSL and to increase our understanding of the mechanism of action of immunomodulators so that their use can be more rationally applied.

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