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Prospective Double-Blind Evaluation of Topical Adenine Arabinoside in Male Herpes Progenitalis

EDWARD L. GOODMAN,* JAMES P. LUBY, AND M. TRACY JOHNSON

*Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas, Texas 75235, and Presbyterian Hospital of Dallas, Dallas, Texas 75231**

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Thirty-four virologically proven episodes of herpes progenitalis in 32 men were treated in a prospective double-blind study with either adenine arabinoside ointment or an identical-appearing placebo for 7 days. Clinical evaluation and quantitative virological studies were done on days 1, 3, and 8. There was a highly significant correlation between clinical response and quantitative virology. There was no difference in clinical or virological response between drug and control groups. Primary attacks tended to have higher viral excretion over the period of observation. The level of complement-fixing antibody to herpes simplex virus type 2 (<1:16 versus ≥1:16) in patients with recurrent disease did not appear to alter the course of viral excretion.

Herpes progenitalis is a common venereal disease that is caused primarily by herpes simplex virus type 2 (HSV-2). The disease is characterized by recurrent painful genital vesicles and ulcers. The patient is highly infectious when the lesions are active. In addition, the virus can be transmitted from mother to neonate, resulting in disease with high morbidity and mortality. Recent evidence has implicated HSV-2 as a possible factor in the pathogenesis of cervical dysplasia and carcinoma (9).

There is currently no method of treatment for genital herpes. A widely used form of treatment is topical application of neutral red or other heterocyclic vital dyes and exposure to light (3). However, resistance of HSV to dye and light treatment may develop (T. W. Chang, N. Fiumara, and L. Weinstein, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 39, 1974), and Rapp has raised the objection that this form of therapy may actually increase the oncogenic potential of the virus (13). In the series of patients treated by Felber et al., only 14 had genital or perigenital lesions; a good response occurred in eight of nine treated and three of five control patients (3). A recently reported double-blind controlled study failed to demonstrate any efficacy of neutral red dye and light in patients with recurrent herpes simplex infections (8b).

Recent interest in adenine arabinoside (Ara-A) for the parenteral therapy of serious deoxyribonucleic acid virus infections (2, 6) and its established efficacy in the topical therapy of ocular herpes simplex prompted this study (12). Previous data have shown that the median and mean minimal inhibitory concentrations of Ara-A against strains of HSV-2 approximate 20 and 16.6 μg/ml, respectively (4, 8a). The study reported here compares the efficacy of topical Ara-A ointment in a double-blind prospective study of male herpes progenitalis. In the course of performing this study, observations were also made on the natural history of the disease as measured by quantitative viral excretion and on the course of recurrent disease as influenced by complement fixing antibody levels at the time of initial presentation.

MATERIALS AND METHODS

Overall study design. Men with active genital herpes were referred to us by their personal physicians. Written informed consent was obtained. The patients were treated in a randomized double-blind fashion with an ointment containing 3% Ara-A (30,000 μg/ml) in a vehicle containing petrolatum (USP 60%) and mineral oil (USP 40%) or an identical-appearing placebo containing petrolatum and mineral oil at the same concentrations. The patients were instructed to apply the ointment to the lesions four times daily for 7 days. Viral cultures were obtained pretreatment (day 1), the morning of day 3, and the morning of day 8. The patients bathed on the morning before being seen and did not apply ointment so as to preclude apparent sterilization of the lesions by residual drug. On each of the three office visits, a protocol was completed evaluating the condition of the lesions and the extent of pain and noting the presence of any new lesions in the treated area. On day 1 each patient was bled for determination of HSV antibody titer.

Quantitation of clinical response. Emphasis was placed on the change in the patients' status between

office visits. The following were done on days 3 and 8. (i) The condition of the lesions was noted: 3 points were assigned if the condition of the lesions had worsened, 2 points if it was unchanged, 1 point if it improved, as demonstrated by drying or scabbing, and 0 points if the lesions had healed. (ii) The extent of pain was evaluated: 3 points were assigned if more pain was present, 2 points if the amount of pain was unchanged, 1 point if there was less pain, and 0 points if pain was absent. (iii) The presence of any new lesions in the treated area was determined: 4 points were assigned for the presence of new lesions and 0 points for the absence of new lesions. The total clinical score was the sum of points on days 3 and 8, with the maximal score being 20.

Virological methods. Viral cultures were obtained by rolling a premoistened swab over the entire surface of ulcerating lesions or vesicles that had been freshly unroofed. The swabs were then placed in 2 ml of Eagle minimum essential medium with 2% fetal calf serum (transport medium). Cultures were immediately processed by filtering the transport medium through a 0.45-μm filter, making serial 10-fold dilutions, and inoculating 0.1 ml of each dilution into one set of tissue culture tubes containing monolayers of human embryonic lung diploid fibroblasts. Tissue cultures were read daily and recorded as positive or negative for cytopathic effect. At 1 week the cultures were discarded except for a single tube with each patient's isolate, which was frozen at −70 C for later serotyping. Fifty percent tissue culture infectious dose ($TCID_{50}$) end points were calculated by the formula of Reed and Muench (14), and results were expressed as $\log_{10}$ $TCID_{50}$/0.1 ml of transport medium.

Typing of isolates was performed by the quantal microneutralization method of Pauls and Dowdle with constant virus and varying serum (11). Standard strains of HSV-1 and HSV-2 were kindly provided by Andre J. Nahmias, Atlanta, Ga., and antisera were produced in rabbits. Microtiter CF antibody titers were performed by the Laboratory Branch CF method on each patient's serum against crude HSV-1 and HSV-2 antigens (1, 10).

Statistical analyses. Linear regression was performed by the least-squares method. Comparisons were determined by the two-tailed t test. Contingency tables were analyzed by chi-square.

RESULTS

Forty-five episodes of herpes progenitalis were studied in 42 men between January and December 1974. Thirty-four episodes in 32 men were virologically confirmed and constitute the subject matter of this report. Twenty-nine of the viral isolates were tested and typed as HSV-2 by quantal microneutralization studies.

Table 1 lists a comparison of drug- and placebo-treated groups with regard to various parameters of their disease. By history, seven episodes were regarded as primary, whereas 27 represented recurrent disease. Overall, patients with primary attacks tended to be younger and were seen later in their course than those with recurrent episodes. Although the placebo group was first treated slightly later after onset and had a higher geometric mean HSV-2 CF antibody titer than their drug-treated counterparts, these differences were not statistically significant. There was a close similarity between the two groups with regard to the other parameters analyzed. Noteworthy is the correspondence between groups in the amount of virus culturable on day 1.

TABLE 1. *Comparison of drug and placebo treatment groups*

Parameter	Drug	Placebo
Primary attacks	3	4
Recurrent attacks	12	15
Duration of recurrent disease (months)	26.6 ± 6.6[a]	17.9 ± 3.8
Age of patients		
Primary attacks	24.3 ± 1.8	32.3 ± 5.6
Recurrent attacks ...	34.3 ± 2.9	36.6 ± 3.0
Days of lesions before treatment		
Primary attacks	6.7 ± 1.5	10.0 ± 2.3
Recurrent attacks ...	2.0 ± 0.4	4.3 ± 1.3
Geometric mean titer of HSV-2 CF antibody		
Primary attacks	2.0	12.6
Recurrent attacks ...	6.8	13.3
$\log_{10}$ $TCID_{50}$ HSV on day 1		
Primary attacks	2.7 ± 0.2	2.0 ± 0.9
Recurrent attacks ...	2.4 ± 0.3	2.7 ± 0.3

[a] Mean ± standard error of mean.

There was a statistically significant relationship between total clinical point score and the change in viral titer from day 1 to day 3 in 30 episodes for 28 men in whom full quantitative virological and clinical data are available ($P < 0.001$) (Fig. 1). There was, however, no relationship between "response," arbitrarily defined as a clinical point score ≤ 4 and a fall in viral excretion ≥ 1.5 $\log_{10}$ $TCID_{50}$ and whether the patients were treated with drug or placebo. The lack of relationship between response and drug therapy was true for all episodes, for both primary attacks and recurrences when each category was analyzed separately.

Although primary and recurrent episodes had quantities of virus in lesions that were nearly identical on day 1, by day 3 the difference was statistically significant between the two groups ($P < 0.01$). There was, however, no statistically significant difference in quantitative viral excretion for either primary or recurrent episodes between drug or placebo treatment groups (Fig. 2). In those persons with

recurrent disease in whom the CF antibody level to HSV-2 was known, there was no statistically significant difference in quantitative viral excretion according to CF antibody titer. Various combinations were tested (<1:8 versus ≥1:8, <1:16 versus ≥1:16, etc.). Less than 1:16 versus ≥1:16 was representative of the analysis and is shown in Fig. 3.

DISCUSSION

From this double-blind prospective study, it is clear that topical Ara-A did not modify the course of herpes progenitalis in men. The results of this study are striking when a comparison is made between the concentration of drug in the ointment and intimately in contact with the lesions (30,000 μg/ml) and the median and mean minimal inhibiting concentrations of Ara-A against HSV-2 (20 and 16.6 μg/ml). Several explanations may exist for this striking disparity between in vitro efficacy and clinical results:

(i) Ara-A is known to be relatively insoluble, and this may limit its diffusion into the lesion. It may be that a more soluble form of Ara-A, e.g., Ara-A monophosphate, or the use of a different vehicle may be effective in the treatment of herpes progenitalis. The results of this study contrast with the results found in herpetic keratoconjunctivitis, where Ara-A at the same concentration has been shown to be effective (12). Similarly, idoxuridine as 0.5% ophthalmic ointment has also been proven to be effective in the therapy of herpetic keratoconjunctivitis (5), but it is ineffective in the treatment of herpes labialis (8). The use of a different vehicle to enhance therapeutic response is illustrated by a double-blind controlled study showing the more rapid resolution of herpes zoster after application of 40% solution of idoxuridine in dimethyl sulfoxide (7). Dimethyl sulfoxide is not available for use in the United States.

(ii) A second, less likely explanation may be that the herpetic lesion is the result of continuous migration of viral particles from sacral ganglia, and any topical agent may be ineffective in inhibiting this constant influx.

The present study points out the potential utility of quantitative virological techniques in assessing new modes of treatment. Clinical scores correlated closely with changes in viral titers, and the quantitation of virus added to the objective evidence produced in this study. Since the placebo-treated group had had their disease for a longer period of time when first seen and had a higher geometric mean titer of HSV-2 CF antibody, we cannot exclude the possibility that this trial was inadvertently biased against the drug. This, however, seems unlikely since the differences in the constitution of the drug and placebo groups were not statistically significant and since the quantities of virus in the lesions were nearly identical on day 1 and continued to be similar through the study period.

The fact that the level of CF antibody to

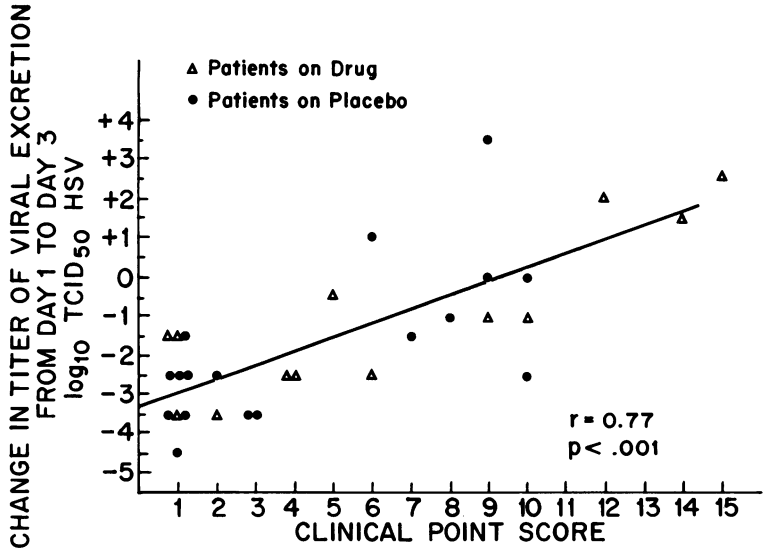

FIG. 1. *Change in quantitative viral excretion from day 1 to day 3 versus total clinical point score in 30 episodes from 28 patients. Viral excretion expressed as $\log_{10}$ $TCID_{50}$ of HSV/0.1 ml of transport medium.*

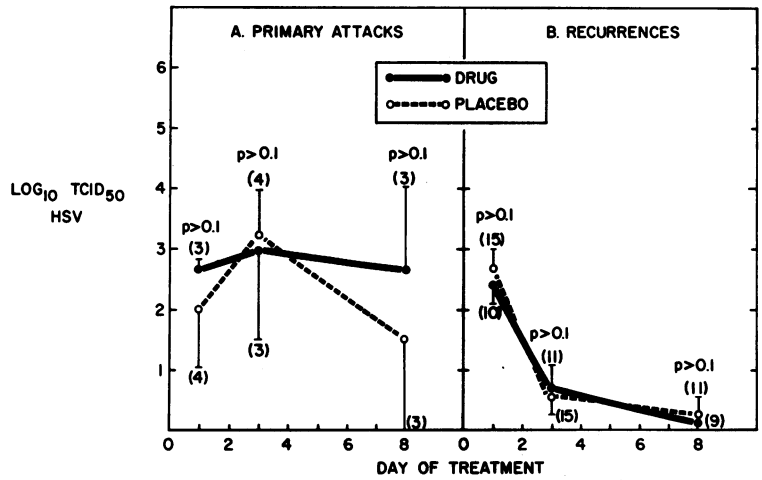

FIG. 2. *Quantitative viral excretion from day 1 through day 8 in the two treatment groups. (A) Primary attacks; (B) recurrences.*

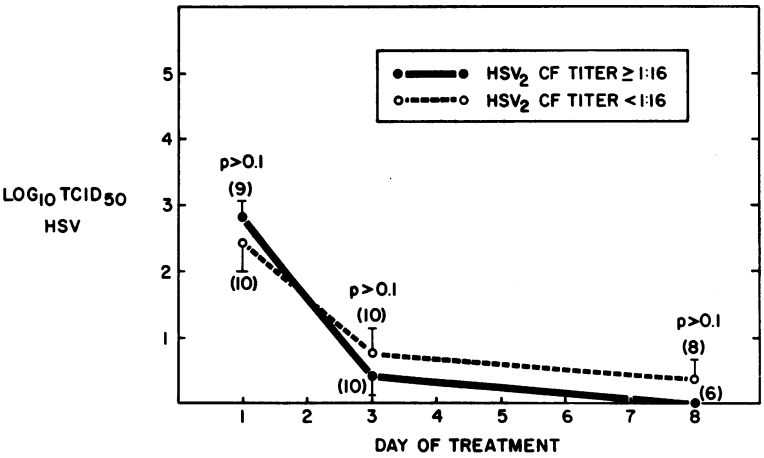

FIG. 3. *Quantitative viral excretion from day 1 through day 8 by CF antibody titers in 20 patients with recurrent disease.*

HSV-2 did not correlate with the course of viral excretion in recurrent disease suggests that therapy directed toward increasing antibody titer alone might not be effective. However, the striking difference in viral excretion between primary attacks and recurrences noted on day 3 of observation (Fig. 2) suggests that in some way previous experience with the virus modifies the course of subsequent attacks, perhaps as a manifestation of cell-mediated immunity.

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