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ARABINOSYLADENINE MONOPHOSPHATE IN GENITAL HERPES: A DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY

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A double-blind, placebo-controlled study was performed in 55 male patients with recurrent herpes simplex genitalis. The 29 patients who received topical arabinosyladenine monophosphate (ara-AMP) showed no significant difference in viral shedding, duration of pain, healing time or development of new lesions as compared to 26 placebo-treated patients. Ara-AMP was well-tolerated when topically applied. Serum neutralizing antibody titers did not change significantly during the acute and convalescent periods of the patient's recurrent HSG attacks. We conclude that ara-AMP, when applied topically as a 10% gel five times a day within 24 h of onset of recurrent HSG, does not influence the virologic and clinical evolution of the recurrent episode.

arabinosyladenine monophosphate genital herpes double-blind, placebo-controlled study

INTRODUCTION

Genital herpes is a major sexually transmitted disease (STD), being more frequently diagnosed than gonorrhea in women visiting private gynecologists [2,4,8]. Recently, data analyzed from the National Disease Therapeutic Index showed that from 1966 to 1979 there was a nine-fold increase in patient visits to private practitioners for consultation concerning this disease [21]. Of great concern to public health officials, physicians, patients and researchers is the fact that herpes simplex genitalis (HSG) is the only major STD without a cure.

A few antiviral agents against herpes simplex virus (HSV) have reached clinical trials within the last 10 years but none has shown definite therapeutic value. There are conflicting data concerning the effectiveness of topically applied idoxuridine for the treatment of herpes simplex labialis (HSL) and herpes simplex genitalis (HSG) [6,10–13, 16,20,23,24,27]. Therapeutic trials using topical arabinosyladenine for HSG in humans have been ineffective [1,9]. 2-Deoxy-D-glucose was reported effective for HSG in humans, but this data has not been substantiated [3]. Vidarabine-5′-phosphate (ara-AMP) is an analog of the purine nucleoside arabinosyladenine possessing antiviral activity against

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herpes simplex virus (HSV) in vitro and in animal models [5,14,15,18,22]. Ara-AMP has antiviral, pharmacologic and toxicologic properties similar to those of the parent compound [25]. Unpublished observations (Dr. Meyer-Rohn, personal communication) suggest that ara-AMP might be effective in the treatment of HSG. We report here the results of our independent evaluation of ara-AMP in HSG and the results indicate that ara-AMP was not effective, confirming the results of a previous trial on its effectiveness in HSL [26].

MATERIALS AND METHODS

Patient population

The patients in this study were all male outpatients referred by private practitioners and the dermatology clinics at New York University Medical Center. Detailed medical histories were taken including information regarding initial genital herpes attack, frequency of recurrences, previous history of HSL and symptoms related to clinical episodes.

All patients were at least 18 years of age and were required to give informed consent prior to enrolling in the study. All recurrent HSG patients had to have pre-enrollment positive viral cultures and primary HSG patients needed pre-enrollment positive Tzanck smears.

Excluded from this study were patients using other forms of antiviral therapy, vaccines, antibiotics or photodynamic inactivation within 2 weeks preceding or during the study period. Also excluded were patients with secondary infections of their herpetic lesions and those patients having other sexually transmitted diseases.

Study design

Patients were stratified into either primary or recurrent genital herpes groups and randomized to receive either a 10 g tube containing 10% ara-AMP in a water-soluble gel base or the gel base alone. Recurrent disease patients were included only if they had typical lesions not more than 24 h old and primary disease patients had to have new lesions not more than 4 days old. The patients rubbed 0.5 inch of gel (30–37 mg) into the affected area five times daily for 7 days. The patients were evaluated on days 1, 3, 5 and 7 for: 1) progression of old lesions; 2) development of new lesions; 3) pain/itching resolution; 4) systemic involvement (fever, lymphadenopathy); 5) virus isolation from the lesions; and 6) acute and convalescent neutralizing antibody titers. All viral cultures were taken from lesions at least 2 h after the gel was last applied and only after cleansing the affected area with alcohol to avoid carry-over of residual drug to the tissue culture. New lesions were defined as lesions appearing within 1 cm of the initial vesicles noted on day 0. Patients were seen by the same physician during each clinic visit.

The number, stage and location of individual lesions were recorded during each evaluation. Adverse reactions unrelated to the natural course of the disease were monitored.

Virology

Genital lesion samples were obtained using cotton tip swabs and were immediately placed in Hank's balanced salt solution. These specimens were inoculated within 2 h onto human foreskin fibroblast cell cultures (FS-7) and were monitored for 7 days for the development of virus-induced cytopathic effect (CPE). Viral titers were determined by a plaque assay in Vero cells on all positive cultures by using the original specimens which were stored at -70°C.

Sera obtained on day 0 and 3 weeks after ending treatment were assayed for the presence of neutralizing HSV-1 and HSV-2 antibodies. All sera were heat-inactivated for 1 h at 56°C. Serial dilutions of sera were mixed with equal volumes of HSV-1 and HSV-2 (200 p.f.u.), incubated for 1 h at 37°C and inoculated into duplicate wells of microtiter plates with confluent layers of FS-7 cells. The highest serum dilution which completely protected 50% of the cells from virus-induced CPE was taken as the serum antibody titer (expressed as $\log_{10}$ units of the reciprocal of the dilution).

Data analysis

The chi-square statistic and p value were used for determining statistical significance between results obtained for the placebo and ara-AMP treatment groups. Patients with primary genital herpes were not analyzed because of their small number.

RESULTS

Comparison of treatment groups

The study group consisted of 59 male patients, 55 recurrent and four primary HSG patients. All these patients complied with our instructions for use of the medication and return visit evaluations. Thirty-one patients were treated with ara-AMP, two with primary disease and 29 with recurrent disease. Twenty-eight patients were treated with placebo, two with primary disease and 26 with recurrent disease. The historical data derived from the 59 patients indicate that the ara-AMP and placebo groups were similar in age and number of years with recurrent genital herpes. There were two blacks, one hispanic and 56 white subjects. Six patients (19.4%) treated with ara-AMP and 11 (39.3%) treated with placebo had an initial negative viral culture. In patients with recurrent disease, the median duration of lesions before beginning treatment was 20 h for those treated with ara-AMP and 18 h for those treated with placebo. The median number of lesions before the initiation of treatment in patients with recurrent disease was 6.0 for both the ara-AMP and placebo treated groups. The majority of patients with recurrent disease had no pre-enrollment pain: 62.1% (18 patients) in the ara-AMP group and 57.7% (15 patients) in the placebo groups. The median duration of pain before beginning treatment was 12 h in the ara-AMP group and 24 h in the placebo group.

Effect of ara-AMP on the clinical and virologic courses of the disease

Pain and itching resolution, defined as no pain/itching or a decrease in pain by days 2 and 3 and without a return to the initial state of pain on a subsequent day, was not significantly different between the two treatment groups as shown in Table 1 ($P = 0.430$). Pain and itching were analyzed only for those 32 patients who experienced pain at some time during the study period. Table 2 shows that there was no statistically significant association between the development of new lesions and treatment (P=0.599): in the ara-AMP-treated group the median number of new lesions was 2.5 (range 1–6) compared to 3.0 (range 1–18) in the placebo group. Using Kaplan–Meier estimates of the healing curves, there is no difference in healing times of patients treated with ara-AMP and those treated with placebo. Investigator assessment of therapeutic effect among treatment and placebo subjects was not significant (P=0.284).

Table 3 shows the elimination of virus from old lesions, defined as a negative viral culture in old lesions on days 2, 3 or 4 with no return to a positive viral culture on a subsequent day. Patients who had a negative viral culture (day 0) are excluded from the analysis. There was no difference between placebo and ara-AMP-treated patients in the disappearance of virus from lesion specimens (P=0.237). There was also no significant difference in viral titers between placebo and ara-AMP-treated patients (data not shown).

TABLE 1

Pain/itching resolution[a] according to treatment group

Treatment group	No. of patients	Improved	No change/worse
Ara-AMP	15	10 (67)[b]	5 (33)
Placebo	17	9 (53)	8 (47)

$\chi^2 = 0.622; P = 0.430$.

[a] See text for definition.

[b] Percentage in brackets.

TABLE 2

Tabulated results of development of new lesions[a] according to treatment group

Treatment	No. of patients	Developed new lesions	
		Yes	No
Ara-AMP	31	12 (39)[b]	19 (61)
Placebo	28	9 (32)	19 (68)

$\chi^2 = 0.276; P = 0.599$.

[a] See text for definition.

[b] Percentage in brackets.

TABLE 3

Elimination of virus from old lesions[a] according to treatment group

Treatment group	No. of patients	Virus culture	
		Negative	Positive
Ara-AMP	25	19 (76)[b]	6 (24)
Placebo	17	10[c] (59)	7 (41)

$\chi^2 = 1.397; P = 0.237$.

[a] See text for definition.

[b] Percentage in brackets.

[c] One patient who did not have a culture done on days 2, 3 or 4, but had one done on day 5 is included.

With the exception of one patient, all subjects were infected with HSV-2 strains. None of the placebo or ara-AMP patients with recurrent HSG showed a statistically significant change in neutralizing antibody titers for HSV-2 as judged from acute and convalescent sera. Three primary HSG patients showed four-fold or greater increases in their antibody titers, although no comparison between placebo and ara-AMP-treated subjects could be made because of the small number of primary patients treated. One primary disease patient failed to return for convalescent sample. It is noteworthy that 90% of all patients had significant titers to HSV-1, indicating previous infection.

Adverse effects of ara-AMP

Overall, there were few, if any, ara-AMP attributable adverse reactions. Five patients treated with ara-AMP experienced transient burning, itching and/or redness in the treated skin area. The drug might have been responsible for these reactions in one patient and possibly involved in three other patients. One patient treated with placebo experienced itching at the site of treatment. An additional patient treated with placebo experienced non-drug attributable diarrhea, sore throat and fever.

DISCUSSION

This is the first double-blind, placebo-controlled clinical trial of ara-AMP in recurrent genital herpes. The data showed that there was no statistically significant difference between ara-AMP-treated patients and placebo-treated patients as assessed by pain resolution, development of new lesions, disappearance of virus in old lesions, investigator-assessed therapeutic effect, and healing time of old lesions.

However, as shown in Table 4, there appears to be a mild antiviral effect between days 2 and 4 as judged by the number of patients having negative cultures: 74% in ara-AMP-treated patients as compared to 57% in the placebo-treated group. However, this difference is not significant.

TABLE 4

Tabulated results of serial virus cultures[a] according to treatment group

Treatment group	Pretreatment		Day 1		Days 2–4		Days 5–7	
	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Ara-AMP	25 (81)[c]	6 (19)	17 (55)	13 (42)	7 (23)	23 (74)	0 (0)	11 (36)
Placebo	17 (61)	11 (39)	22 (79)	5 (18)	10 (36)	16 (57)	1 (4)	14 (50)

[a] Results from specimens were collected only from initial lesions. No specimens were collected after healing of lesions.

[b] If a patient was observed on more than one of these days, the last observation was used in constructing the table.

[c] Percentage in brackets.

Ara-AMP was well tolerated by the group as a whole. The one patient who definitely reacted adversely to the medication has a history of irritant reactions to Stoxil ® ointment as well.

Spruance et al. [26] concluded in their study of 233 patients with recurrent HSL that ara-AMP was ineffective because it could not penetrate the skin in order to inhibit viral replication. We feel that lack of penetration is not a completely satisfactory explanation for the failure of ara-A and ara-AMP in the treatment of recurrent disease. Other studies support the same conclusion [15,18], and we feel that these drugs display only minimal inhibitory action on virus replication in recurrent lesions when applied topically. Indeed, it was shown that HSV-induced skin infections in hairless mice respond in a similar way to ara-A cream and ara-A dissolved in dimethyl sulfoxide, which easily penetrates deeply into the skin [19]. On the other hand, previous results suggest that systemic administration is more effective than topical application of ara-A and ara-AMP in the treatment of primary experimental HSV infections [14,17].

Despite the relative small number of patients, the results of ara-AMP in HSG confirm previous results with other drugs and other forms of recurrent herpes. It appears that these drugs do not improve to any significant extent the clinical evolution of recurrent herpes. In addition, these drugs have no effect on the latent virus and we agree with the statement of Field et al. [7] that 'attempts to eradicate the latent infection during the quiescent phase are likely to be fruitless and these drugs should not be used for this purpose'.

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