238. Biopsies from 91 patients with diagnosis other than CSD all stained by the Warthin-Starry method did not reveal the presence of CSD organisms.
9. R. C. Brown and H. C. Hopps, [*Am. J. Clin. Pathol.* **60**, 234 (1973)], modified as follows. See p. 237, 3a: Chroma-Gesellaschaft basic fuchsin (1 g per 100 ml); may stir overnight, filtered in Nalgene sterilization filter unit type S. See p. 238: step 2, 1 minute; step 4, 1 minute; step 6, delete; step 8, if background is not clear at end of step 8, repeat steps 4, 5, 7, and 8; step 10, Gallego's solution, two changes, 1 minute each; step 12, delete blot; step 13, acetone 30 seconds; step 14, in solution for 2 to 3 minutes; step 15, delete; step 16, in acetone-xylene, two changes; step 17, delete; step 18, two changes. See Notes: slides are placed in staining racks for steps 2 to 12, and in staining dish for steps 13 to 18.
10. E. Heyferman, *J. Clin. Pathol.* **32**, 971 (1979). The patient's serum (antibody) and the rabbit antiserum to human immunoglobulin conjugated with horseradish peroxidase were added to the bacteria (antigen) in a tissue section. Addition of the substrate caused the formation of a black precipitate. For the immunoperoxidase staining we used the Vectastain ABC procedure (Vector Laboratories, Inc.).
11. One of us (A.M.M.) has used CSD skin test antigens (MG, JC) to test 143 patients for the diagnosis of CSD (118 positives, 25 negatives) from 1981 to present.
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13. We thank D. H. Connor and D. R. Wilson for help with the manuscript and T. C. Allen and N. A. Powers for technical assistance. We also thank D. R. Bernier for providing hyperimmune rabbit sera to rickettsial agents and slides containing *Rickettsia*-infected tissue culture cells.

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Carbocyclic Arabinofuranosyladenine (Cyclaradine): Efficacy Against Genital Herpes in Guinea Pigs

Abstract. *Carbocyclic arabinofuranosyladenine (cyclaradine), a novel nucleoside analog with such desired features as hydrolytic and enzymatic stability, adenosine deaminase resistance, and low systemic toxicity, inhibited the replication of herpes simplex virus types 1 and 2. The 5′-methoxyacetate prodrug form exhibited significant efficacy in the topical treatment of genital infections by herpes simplex virus type 2.*

Until recently it was virtually agreed that viral diseases do not respond to chemotherapy. This belief is vanishing as new agents are developed that are effective against specific viral infections. Among the most promising agents to date are the nucleoside analog 9-β-D-arabinofuranosyladenine (Ara-A) (*1, 2*) and 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir) (*3*). Both drugs have serious drawbacks inherent in their molecular structural design that limit their use in the treatment of herpes simplex virus (HSV) infections, especially HSV-2 genital infections. Thus Ara-A therapy has not been successful in the topical treatment of experimental mucocutaneous HSV infections in animals (*4*) or against oral or genital HSV infections in humans (*5*). The molecular features of the Ara-A molecule are compatible with the substrate requirements of the ubiquitous enzyme adenosine deaminase, and it is generally accepted that the major liability in its effectiveness is rapid deamination to the much less active arabinosyl hypoxanthine (*2*). Clinical studies have demonstrated that topical acyclovir is of minimal benefit in the treatment of recurrent genital herpes (*6*). HSV strains that are deficient in their ability to code for thymidine kinase are resistant to the drug (*7*).

We describe here a novel nucleoside analog that is highly efficacious in the topical treatment of HSV-2 genital infections. Carbocyclic Ara-A (cyclaradine), an adenosine deaminase–resistant analog of Ara-A, inhibited the replication of HSV-1 (strain HF) and HSV-2 (strain MS) in tissue culture at noncytotoxic concentrations (*8*). Preliminary detection of antiviral activity was determined by measuring its ability to inhibit virus-induced cytopathogenic effects in infected cultures (*9*). Cyclaradine was active against acyclovir-resistant HSV variants deficient in DNA polymerase or thymidine kinase activities (*10*). This finding indicates that the carbocyclic nucleoside is not activated by virus-induced thymidine kinase in infected cells. Optimum derivatization and formulation of cyclaradine to the methoxyacetate ester prodrug form (cyclaradine-MA) yielded a new topically active antiherpes agent (Fig. 1).

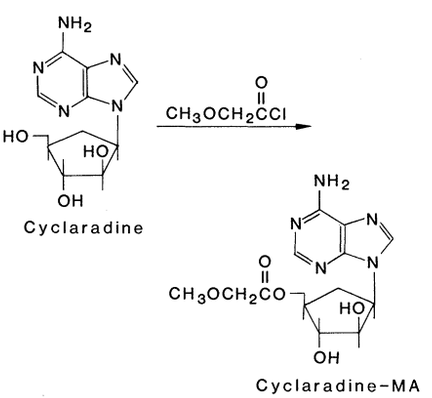

Fig. 1. Preparation of cyclaradine-MA. To a solution of cyclaradine (0.10 mole) in dimethylformamide (750 ml) was added methoxyacetyl chloride (0.11 mole) in dimethylformamide (140 ml). The reaction mixture was stirred at 4°C overnight and then water (50 ml) and sodium bicarbonate (0.27 mole) were added. The volatile materials were removed in vacuo and the residue was applied to a silica-gel column. Elution of the major fraction with methanol-chloroform (0.15 per liter) gave the pure product (melting point 172° to 174°C). Crystallization from water and subsequent drying in vacuo at room temperature gave the dihydrate (melting point 78° to 80°C).

Cyclaradine belongs to a class of compounds known as carbocyclic nucleosides, in which a methylene group replaces the oxygen atom of the carbohydrate ring (*11*). Earlier studies showed that these analogs, which lack the labile glycosidic bond, are stable to cleavage by phosphorylases or hydrolases while retaining the potential for therapeutic useful interaction with other enzymes involved in nucleotide metabolism (*8, 12*). In the case of cyclaradine, the exchange of methylene for oxygen also renders the compound inert to adenosine deaminase. Thus, under conditions in which Ara-A is completely deaminated (1 μmole/min per unit of enzyme) by calf intestinal adenosine deaminase (type III, Sigma), no detectable deamination of cyclaradine was observed. As expected, the addition of the adenosine deaminase inhibitor, 2′-deoxycoformycin, to growing P-388 mouse lymphoid leukemia cells increased the cytotoxicity of Ara-A 20-fold, while no increase in toxicity was observed with cyclaradine.

The effect of the compounds on genital herpes infections was studied in guinea pigs, an animal model with many human-like parameters. Beginning 2 to 3 days after the intravaginal inoculation of guinea pigs with 10^5 median cell culture infectious doses per 0.1 ml of HSV-2 [strain MS (*13*)], typical discrete vesicular lesions appeared on the external genitalia in 98 percent of the animals (Fig. 2). Peak mean lesion scores (*14*) 7 days after infection were 3.53 and 3.35 in HSV-infected untreated and placebo-treated control groups, respectively.

Initial examination of the parent compound, cyclaradine, indicated that the unaltered nucleoside analog is not suitable for topical treatment of genital HSV-2 infections. It was subsequently observed that the low solubility of the agent caused precipitation from the gel, and the crystalline material appeared to remain at the surface of the vaginal mucosa. Unlike cyclaradine and its simple alkyl ester derivatives, certain alkoxyalkanoate esters were easily formulated into more amorphous-like materials that could be easily applied topically to the guinea pig. The 5′-methoxyacetate (cyclaradine-MA) was selected for subsequent studies.

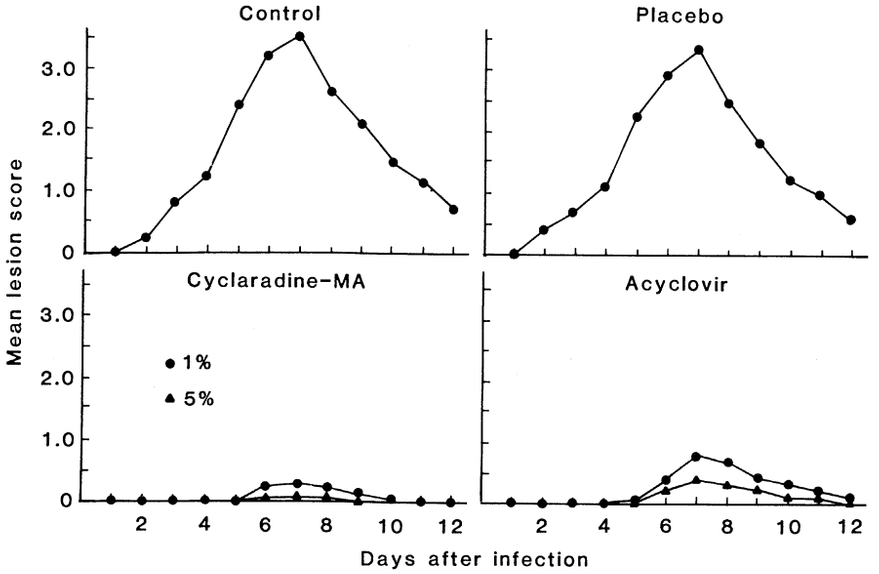

Fig. 2. Effect of topical treatment with cyclaradine-MA and acyclovir on the severity of HSV-2 genital lesions in guinea pigs. Hartley albino female guinea pigs were inoculated intravaginally with 10^5 median cell culture infectious doses of HSV-2 (strain MS) suspended in 0.1 ml of cell culture medium. All compounds were suspended in 0.4 percent agarose gel and administered intravaginally in a volume of 0.1 ml three times daily for 7 days starting 3 hours after infection. There were 20 animals in each control group and 10 in each drug-treated group. The placebo consisted of the 0.4 percent agarose gel alone. Lesions appearing on the external genitalia were scored on a scale of 0 to 4 (*14*).

When administered intravaginally as 1 or 5 percent gels, cyclaradine-MA (given three times daily for 7 days, starting 3 hours after infection) was found to significantly suppress lesion development (Fig. 2). Only one animal in the 1 percent group developed lesions, which appeared on day 6. The peak lesion score 7 days after infection was 0.30 for this drug-treated group, compared with 3.35 and 3.53 for the HSV-infected control groups. Only one of the animals treated with 5 percent cyclaradine-MA exhibited redness and swelling, and these symptoms never progressed to actual lesion development. Hence, the peak lesion score for this group was 0.05. The mean virus titers of the drug-treated groups were significantly below the mean virus titers of the control groups (*15*).

Intravaginal treatment of virus-infected guinea pigs with 1 and 5 percent gels of acyclovir (administered three times daily for 7 days, starting 3 hours after infection) also suppressed lesion development, but not as markedly as did cyclaradine-MA (Fig. 2). In the acyclovir-treated groups, 20 to 30 percent of the animals exhibited typical HSV-2 genital lesions, with peak lesion scores of 0.80 for 1 percent acyclovir-treated animals and 0.35 for 5 percent acyclovir-treated animals.

Recurrent lesions developed in the control groups and in the acyclovir-treated groups between 19 and 24 days after infection (12 to 17 days after the end of treatment). No evidence of recurrent infection or new lesion development was observed at any time for the cyclaradine-MA–treated groups. No toxicity of skin irritation was observed in any of the drug toxicity control groups treated intravaginally with cyclaradine-MA during the experiments (*16*).

These results suggest that optimum derivatization and formulation of cyclaradine may result in a topically useful new antiherpes drug. The high antiviral activity in comparison with other antiherpes compounds, and such desirable features as hydrolytic stability, adenosine deaminase resistance, and low systemic toxicity (*17*), make cyclaradine and its derivatives excellent candidates for clinical trials against herpes virus infections.

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13. The MS strain of HSV-2 was obtained from E. R. Kern (University of Utah). The virus was propagated and titered in primary rabbit kidney cell cultures in these laboratories.
14. We used the lesion scoring procedure of E. R. Kern, L. A. Glasgow, J. C. Overall, Jr., J. M. Reno, and J. A. Boezi [*Antimicrob. Agents Chemother.* **14**, 817 (1978)]. Virus-induced lesions were scored daily (days 1 through 12) according to the following scale: 0, no apparent evidence of virus infection; 0.5, redness and swelling; 1.0, distinct area where vesicle might be forming; 1.5, single discrete vesicle; 2.0, several discrete vesicles; 2.5, many small vesicles; 3.0, many large vesicles; 3.5, many large vesicles coalescing; and 4.0, large ulcers and maceration. A declining score was used during the healing stage and a mean lesion score for all animals was calculated for each day of observation.
15. Vaginal swabs for isolation of HSV-2 were obtained 1, 3, 5, 7, and 10 days after infection. Swabs were placed in 1.0 ml of cell culture medium containing gentamicin and stored frozen until being assayed for infectious virus with primary rabbit kidney cell monolayer cultures grown in plastic microtitration plates. Geometric mean virus titers were calculated for each group of animals for each of the virus assay days. Samples from uninfected, drug-treated control animals were also tested in vitro for residual antiviral activity against HSV-2. No evidence of cyclaradine-MA in sufficient concentration to inhibit virus replication was ever detected in the vaginal swab samples collected from these uninfected, drug-treated animals. Reductions in mean virus titers corresponded to reductions in mean lesion scores in virus-infected, drug-treated guinea pigs.
16. These experiments have been repeated several times with similar results.
17. No significant weight loss was observed in uninfected mice treated four times a day with cyclaradine at a dose of 750 mg/kg per day for 7 days. On the other hand, significant weight loss was observed in uninfected mice treated with Ara-A at the equivalent dose level (755 mg/kg per day).
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