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Adenine arabinoside effect on experimental idoxuridine-resistant herpes simplex infection. ANTHONY B. NESBURN, CHRISTINE ROBINSON, AND RANDOLPH DICKINSON.

Topical application of idoxuridine (IDU) is presently a standard treatment for epithelial herpes simplex keratitis in man. While IDU therapy is usually satisfactory, clinically resistant keratitis has been encountered. In most instances, the actual basis of these IDU treatment failures is never determined. On occasion, viral isolates from such cases have proved to be biochemically resistant to IDU. An important attribute for any new antiviral drug would be its ability to successfully manage cases in which IDU failed clinically, including those in which the infecting virus was biochemically resistant to IDU.

Adenine arabinoside (ARA-A), a new antiviral agent being actively investigated, has demonstrated its efficacy in suppressing both experimental and previously untreated human herpes keratitis. In addition, we and others have found ARA-A effective in instances where IDU therapy was clinically unsuccessful. In one case where ARA-A appeared to be efficacious and IDU failed, the virus isolated was actually biochemically resistant to IDU.

This study was performed to determine, under controlled experimental conditions, whether ARA-A is effective against the herpes simplex virus (HSV) exhibiting biochemical resistance to IDU. Stable, highly IDU-resistant virus strains were produced by growing McKrae-strain HSV in the presence of increasing concentrations of IDU. Strain I was produced in our laboratory, and Strain II was supplied by Dr. Herbert E. Kaufman. Strain II was used for in vivo studies. In vitro studies were carried out with both strains and no significant difference was demonstrated between them.

In vitro drug sensitivity studies and all titrations were performed using micro-testing plates (Falcon). Virus and drug dilutions were applied simultaneously to primary rabbit kidney cells after aspirating the microplate wells. Four wells were used for each dilution and the experiments done in duplicate. Appropriate cells, virus, and drug controls were included. Following a 16 hour adsorption, the wells were aspirated and refilled with fresh medium containing experimental drug. The wells were read “blind” every day for seven days and were considered negative only if viral cytopathology was completely suppressed. Virus titers, calculated by the Karber method, were expressed as the TCID₉₀ per cent end point.

In tissue culture, McKrae-stained HSV production was suppressed significantly by 40 µg per milliliter or by 100 µg per milliliter of either
drug. At concentrations of 100 μg per milliliter, IDU reduced virus titer by 2.0 logs and ARA-A produced a 3.5 log reduction. By contrast, IDU-resistant virus showed a 4.1 log reduction in titer with ARA-A (100 μg per milliliter). Both strains, I and II, were highly IDU-resistant, requiring a concentration of IDU in excess of 400 μg per milliliter to reduce virus titer by 2 log units.

In vivo efficacy of ARA-A and IDU against keratitis induced by McKrae- and IDU-resistant HSV was tested in New Zealand albino rabbits. In each experiment, both eyes of 24 male rabbits, weighing approximately two kilograms, were infected by inoculating 0.05 ml. of virus suspension (titer: $10^7$ TCID$_{50}$ per milliliter) into the lower cul-de-sac and massaging the lids against the globe for 15 seconds. Infection was repeated in 30 minutes. The procedure produced typical acute herpetic keratitis in all eyes. Eyes were examined daily by the same observer using the slit lamp biomicroscope and 0.25 per cent fluorescein. The examiner was unaware of the treatment being administered. Grading of epithelial (dendritic and geographic) keratitis was based on the estimates of overall area of lesion involvement. A 0 to 4+ grading system was used, divided into quarter-step intervals below 1+ and half-step intervals above 1+. One plus was equivalent to 25 per cent of the corneal area involved; 2+, 50 per cent; 3+, 75 per cent; and 4+, 100 per cent involvement.

Seventy-two hours after infection, when dendritic figures were recognizable, animals were divided into three groups of comparable severity.
Then the groups were randomly assigned to receive one of three treatments. Both eyes of each animal were treated with the same medication. This procedure was followed to minimize the chance of administering the incorrect medication and to eliminate the influence of systematically adsorbed antiviral medication on dissimilarly treated eyes. Treatment consisted of one of the following: 3.3 per cent ARA-A ointment, 0.5 per cent IDU ointment, or petrolatum alba. A one centimeter long strip of appropriate drug was instilled into the lower conjunctival cul-de-sac every four hours for five days.

Results of treating keratitis produced by the parent McKrae strain and by the IDU-resistant herpes virus strain are reported. Fig. 1 shows the effect of ARA-A, IDU, and placebo ointments in McKrae-infected eyes after 48 hours of treatment. Severity scores for keratitis at the beginning of treatment (72 hours after infection) are not shown, but were equal in all groups. The distribution of keratitis ran from one quarter to 3+ with a median of approximately 1+. The figure shows that after 48 hours of placebo therapy, the scores for control eyes clustered at the 3+ to 4+ severity grade, while the drug-treated keratitis scores clustered at the low end of the severity spectrum. Nonparametric data analysis considering each eye as an independent variable indicates both drugs to have a highly statistically significant effect on epithelial keratitis, as compared to placebo (P = 0.001). In this experiment (and others not reported here), the IDU, 0.5 per cent seems to suppress epithelial keratitis slightly more quickly and to a slightly greater extent than ARA-A, 3.3 per cent; however, the difference is not statistically significant (P = 0.10).

Results of the same therapy on keratitis produced by the IDU-resistant strain are strikingly different. In Fig. 2, note that IDU seems to have some effect as compared to placebo treatment after 48 hours, but that effect is not statistically significant (P = 0.10). By contrast, the ARA-A therapy is effective and statistically significant when compared to either IDU therapy (P = 0.01) or placebo therapy (P = 0.001).

These laboratory data demonstrate that ARA-A is effective in vitro and in vivo in treating keratitis slightly more quickly and to a slightly greater extent than IDU. Too few patients have been treated to know how often cases clinically resistant to ARA-A will be encountered. Such cases, including ones caused by virus strains biochemically resistant to ARA-A should be expected. Not surprisingly, we find in vitro that it is almost as easy to select for ARA-A-resistant strains of HSV (unpublished data) as it is to select for the IDU-resistant virus used in this study.

Key words: IDU-resistant herpes simplex virus, keratitis, adenine arabinoside.

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Soluble antigens of the bovine cornea. J. M. Hall, G. Smolin, and F. M. Wilson, II.

The antigenic proteins in soluble extracts of bovine cornea and corneal epithelium were studied by immunoelectrophoresis and by immunodiffusion. The extract prepared from whole cornea contained alpha-, beta-, and gamma-globulins, and an albumin-like protein. The epithelium contained only traces of gamma-globulin and an albumin-like protein. A protein that appeared to be intrinsically corneal was present in both the whole cornea extract and in the epithelial extract.

Soluble corneal proteins have been the subject