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Therapy of Experimental Herpes Simplex Keratitis in Rabbits with 5-Iodo-5'-Amino-2',5'-Dideoxyuridine[1] (39882)

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5-Iodo-5'-amino-2',5'-dideoxyuridine (AIU, AIdUrd), the 5'-amino analog of 5'-iodo-2'-deoxyuridine (IdUrd, idoxuridine) is a potent inhibitor of herpes simplex virus replication in cell culture (1). Unlike IdUrd and other antiviral nucleosides, including adenine arabinoside (ara-A, vidarabine) and 5-trifluoromethyl-2'-deoxyuridine (F_3TDR, trifluorothymidine), AIU produces no detectable cellular toxicity *in vitro,* even when administered at millimolar concentrations (1). AIU is metabolized *in vitro* only by HSV-infected cells and, after conversion to the 5'-triphosphate, is incorporated into both viral and cellular DNA (2). Subsequent studies have demonstrated that the initial phosphorylation of AIU is mediated by the HSV-specific thymidine kinase (3), (M. S. Chen, Y-C. Cheng, W. P. Summers, W. H. Prusoff, and D. C. Ward, manuscript in preparation). In contrast, AIU is not a substrate for, nor an inhibitor of, the normal cellular thymidine kinase enzymes. This pattern of selective inhibition of viral metabolism suggested that AIU might have therapeutic potential for the treatment of herpes simplex infections and exhibit advantages over existing antiviral drugs. Our initial evaluation of the clinical potential for AIU showed that ophthalmic solutions of AIU (at 4–8 mg/ml) were highly effective in the treatment of experimental herpes simplex keratitis in rabbits (4), although not as effective as IdUrd. The experiments reported here demonstrate that AIU can provide as effective a therapy of experimental herpes simplex keratitis as IdUrd when it is administered at higher concentrations in an ophthalmic ointment.

Methods and materials. Drugs. IdUrd was obtained from Calbiochem, LaJolla, California. AIU was synthesized by the method of Lin *et al.* (5). Ophthalmic ointments of IdUrd, 0.5%; AIU, 10%; AIU, 30%; and dextrose, 30% were prepared on a weight-to-weight basis with sterile, white petrolatum. These ointments were identical in appearance and coded by the pharmacist so that the other investigators were not aware of the substance instilled during the course of the experiment.

Animals. A total of 32 New Zealand albino male rabbits weighing 1.5 to 2.0 kg were used. The rabbits were maintained in special quarters with ventilation and isolation techniques designed for the maintenance of animals with infectious diseases.

Virus. The virus used was herpes simplex, type 1, (NIH strain No. 11124). Viral stocks were maintained through multiple passages in cultured Vero cells using an infection multiplicity of 10 plaque-forming units per cell. Between passages, the virus pool was stored at −60° in Hanks basal minimal essential medium supplemented with 50% (v/v) fetal bovine serum. The frozen virus suspension was thawed to 21° and 0.1 ml of the virus solution, containing $10^{3.5}$ infectious units ($TCID_{50}$), was used for inoculation.

Inoculation. Three overlapping circular

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epithelial abrasions were made in the cornea of each animal by touching it with a 6-mm corneal trephine. Care was taken to avoid deep penetration of the stroma. Virus-containing solution, (0.1 ml), was dropped into the lower cul-de-sac of each eye. Following this, the lids were manually closed and rubbed against the eye for 30 sec.

Examination and grading. Each animal was graded daily by two independent observers in the manner described below, and the average of the two grades was recorded. Grading was carried out for a period of 10 days following infection. One drop of fluorescein in a 10% solution was instilled into each eye; the corneas were examined at 3× magnification with an ultraviolet light and using the slit-lamp biomicroscope. The severity of the keratitis was scored on a scale of 0 to 3. The grades represented the following: *Grade 0,* no detectable dendritic ulcers; *Grade 0.5,* 1 to 4 dendritic ulcers limited to the epithelium along the lines of abrasion, with no stromal involvement; *Grade 1.0,* 5 to 9 dendritic ulcers limited to the lines of abrasion, without deep stromal edema beneath the involved epithelium; *Grade 1.5,* 5 to 9 dendritic ulcers which are not limited to the lines of abrasion, with or without deep stromal edema beneath the involved epithelium; *Grade 2.0,* 10 to 20 dendritic ulcers, or a confluent ulcer not exceeding one-third of the surface area of the cornea, with or without deep stromal edema beneath the involved epithelium; *Grade 2.5,* greater than 20 dendritic ulcers, with or without deep stromal edema beneath the involved epithelium; *Grade 3.0,* a confluent corneal ulcer involving over one-third of the corneal surface, with deep stromal edema beneath the involved epithelium.

Treatment. Twenty-four hours following viral infection, the animals were divided into five groups which were matched with regard to severity of the lesions. Treatment was then initiated with the following ophthalmic ointments: (i) control, 30% dextrose; (ii) IdUrd, 0.5%; (iii) AIU, 10%; (iv) AIU, 30%. The ointments were stored in 1-ml syringes and identified only by code. There was no difference in the appearance of the preparations. Neither the individuals treating the animals nor those grading the keratitis knew the code until the experiment was terminated. In a preliminary experiment, using ^{125}I-labeled AIU ointment in the eyes of normal rabbits, significant radioactivity was detected in ocular and adnexal tissues 6 hr after application of the ointment. Ointment (0.1 ml) was placed into the lower cul-de-sac of each eye at 6-hr intervals for 72 hr, for a total of 12 treatments. Each animal received the same medication in both eyes.

Results. At 24 hr following infection, the rabbit corneas possessed, on the average, one to four dendrites, typically along the lines of epithelial abrasion. The average lesion score of all 64 corneas in the study was 0.6, with grades ranging from 0 to 2.0. Treatment was instituted at this time. The mean and cumulative corneal lesion grades were observed during the 10-day observation period are shown in Figs. 1 and 2.

Placebo (dextrose)-treated animals. These animals showed increasingly severe herpetic keratitis, reaching an average severity of 2.5 on the third day after infection. The keratitis remained above 2.0 until the sixth day after infection. Then, over a 24-hr period, the degree of keratitis improved from 2.3 to 1.4. On the tenth and last day of observation, the mean lesion score for this group was 0.8, with 7 of the 16 corneas in this group totally without keratitis.

IdUrd (0.5%)-treated animals. The ker-

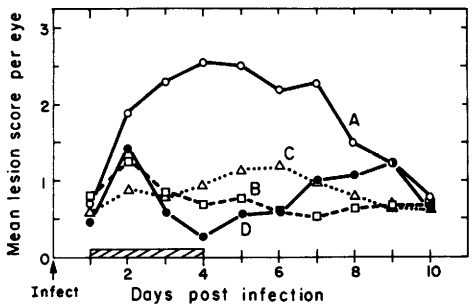

FIG. 1. Mean lesion score per eye as a function of time after infection. Group A = control; Group B = AIU, 10%; Group C = AIU, 30%; Group D = IdUrd, 0.5%. The 72-hr period during which drug therapy was applied is indicated by cross-hatching.

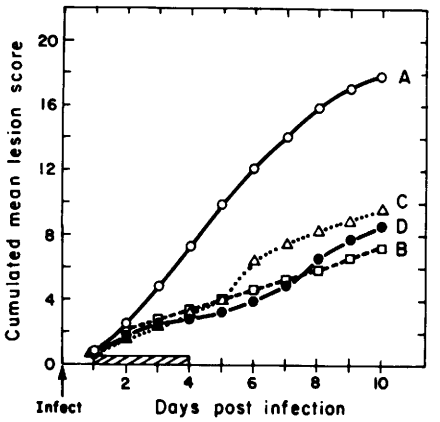

FIG. 2. Cumulative sum of the average daily lesion score per eye as a function of time after infection. Group A = control; Group B = AIU, 10%; Group C = AIU, 30%; Group D = IdUrd, 0.5%.

atitis in these animals worsened during the first day of treatment, increasing from an average pretreatment score of 0.5 to a score of 1.4 after 24 hr of treatment. During the last 2 days of treatment, the keratitis improved, with a score of 0.3 on the final day of treatment. At the end of the treatment period, 7 of 16 corneas were entirely healed. However, in the period after cessation of treatment, there was a marked rebound, with a steady worsening of the keratitis to a mean lesion score of 1.2 on the fifth day after treatment was stopped. On the tenth and last day of study, 5 of 16 corneas were free of disease.

AIU (10%)-treated animals. At the start of treatment, these animals had an average keratitis score of 0.6. The keratitis initially worsened, in a manner similar to the IdUrd and the 30% AIU treatment groups. During the second and third days of treatment, the keratitis improved, with an average lesion score of 0.7 on the final day of treatment. At this time, 4 of 16 eyes were entirely healed, the remainder ranging between grades 0.5 and 1.8. Unlike the IdUrd treatment group, there was no worsening of the keratitis after the cessation of therapy, except on Day 5 when the mean lesion score was 0.8. The average score for the last five days of the study was 0.6. On the last day, 2 of the 16 corneas were entirely healed and an additional 7 had one to four dendrites present.

AIU (30%)-treated animals. This group responded in essentially the same manner as the AIU 10% group. The keratitis in this group of animals worsened slightly during the course of treatment, having advanced from a grade of 0.6 at the initiation of treatment of 0.9 by the third day. The keratitis remained essentially stable on the first 3 days after the cessation of treatment. During the next 3 days, the keratitis score improved; and 5 days after the cessation of therapy, the mean keratitis score was 0.7. At the end of the study, 5 of 16 corneas were entirely free of disease.

Statistical analysis. Data for Days 1 through 6 were analyzed nonparametrically with Mann-Whitney test statistics for two independent variables. Lesion scores for both eyes of any one rabbit were summed. The following relationships were found, based on the eight rabbits in each group. Each of the three drug-treated groups receiving either IdUrd or AIU was significantly different from the placebo (dextrose-treated) controls. There was no significant difference between either group receiving AIU and the IdUrd-treated group.

Discussion. Herpes simplex virus infection is the single most frequent cause of corneal opacities in the developed countries, and appears to be a leading cause of corneal disease in the developing nations as well (6, 7). There are a number of antiviral agents with proven efficacy in the treatment of human herpetic keratitis: IdUrd (idoxuridine); ara-A (vidarabine); F_3TDR (trifluorothymidine); and ara-C (cytosine arabinoside). However, there is a need for alternative antiviral therapy for ocular viral infection. The antiviral action of IdUrd, the most widely used drug in the treatment of dendritic keratitis, is related to the adverse biological consequences of incorporating this thymidine analog into viral DNA (8). The further incorporation of IdUrd into the DNA of normal uninfected cells is most likely responsible for the considerable toxicity that has been found during systemic and topical use of this drug in humans (9, 10). The ocular toxicity of IdUrd is manifested as punctate epithelial keratopathy (the earliest and

most frequent side effect), a follicular conjunctivitis, a contact dermatitis, or narrowing and occlusion of the puncta (10). Moderate to severe toxicity of the regenerating epithelium has been observed during corneal wound healing (11). IdUrd-resistant strains of HSV type 1 have been found (12, 13). An additional concern has been the teratogenicity of IdUrd. This has been demonstrated in pregnant rabbits receiving the drug topically to the eye in doses similar to those used clinically in humans (14).

Ara-A, the other antiherpetic agent currently licensed in the United States for general use, has been shown to be as effective as, but not better than, IdUrd in the treatment of acute dendritic keratitis (15). Ara-A shows significantly less ocular toxicity than IdUrd, although it does show moderate *in vitro* cytotoxicity at antiviral concentrations (1), is teratogenic when administered to pregnant rabbits (16), and has been reported to produce human chromosome breakage *in vitro* and *in vivo* (17, 18). F_3TRD has been shown to be more effective than IdUrd in the treatment of herpetic keratitis, although it has ocular toxicity similar to that of IdUrd (10, 19).

AIU is a new thymidine analog differing from IdUrd by the substitution of an amino group for the 5′-hydroxyl. Initial studies with AIU showed that it was a potent inhibitor of the replication of herpes simplex virus, type 1, in concentrations that were totally devoid of apparent cellular toxicity. At molar concentrations of AIU, ara-A, IdUrd, F_3TDR, and ara-C which produced a comparable degree of inhibition of the replication of HSV in cell culture, only AIU produced no cytotoxicity in the uninfected host cells (1). AIU did not inhibit the replication of any other DNA viruses tested (including SV40, adenovirus, or vaccinia virus); of the RNA viruses tested (i.e., polio, sendai, measles, influenza, Rous sarcoma, and Maloney and Rauscher murine leukemia viruses), only the murine leukemia viruses were inhibited (D. C. Ward, N. H. Ruddle and W. H. Prusoff, unpublished data). Teratologic studies have shown that AIU does not retard growth or induce any developmental defects in neonatal mice, even when administered at dosages as high as 450 mg/kg/day (D. M. Albert *et al.*, unpublished data). IdUrd, in contrast, in doses half as great, produces a marked growth retardation in neonatal mice, and produces developmental abnormalities such as cataract, retinal dysplasia, and retardation of retinal development (20).

Initial studies on the mechanism of action of AIU have shown a pattern of selective inhibition of viral metabolism. Preincubation of HSV-1 with AIU prior to infection does not decrease virion infectivity. When AIU is present in the media only during the adsorption process, no inhibition of virus production occurs. In contrast, addition of AIU (200 μM) 4 to 6 hr postinfection markedly reduces the yield of progeny virus (1). When murine, simian, or human cells in culture are treated with ^{125}I-labeled AIU for up to 24 hr, essentially none of the nucleoside becomes cell associated (2). In contrast, upon HSV-1 virus infection, significant radiolabel is detected in both nucleotide pools and in DNA (2). The major acid-soluble metabolite has been shown by enzymatic and chromatographic analysis to be the 5′-triphosphate of AIU (2).

It has been reported that infection of a cell with herpes simplex virus results in the production of a viral-specific thymidine kinase enzyme (3). Initial experiments have shown that AIU is ineffective in inhibiting viral replication in cells infected with an HSV mutant lacking the virus-specific thymidine kinase enzyme. Subsequent studies have shown that AIU is phosphorylated by HSV-1- and HSV-2-specific thymidine kinase (M. S. Chen, Y. C. Cheng, W. P. Summers, D. C. Ward, and W. H. Prusoff, manuscript in preparation). It is apparent that the inability of uninfected cells to metabolize AIU, which prevents its incorporation into normal host DNA, could account for AIU's striking lack of cellular and systemic toxicity.

The experiments reported here show that AIU is equally effective as IdUrd in the treatment of experimental herpetic keratitis. AIU's potent *in vivo* antiviral efficacy, coupled with its striking lack of

systemic or cellular toxicity and teratogenicity, suggest that further investigation of its systemic and topical use in the treatment of ocular and other herpetic infections is justified.

Summary. AIU (5-iodo-5′-amino-2′,5′-dideoxyuridine, AIdUrd) has been shown to be as effective as IdUrd in the treatment of experimental herpes simplex keratitis in rabbits. AIU's striking lack of cellular or systemic toxicity or teratogenicity and its specific inhibition of viral metabolism are discussed.

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1. Cheng, Y. C., Goz, B., Neenan, J. P., Ward, D. C., and Prusoff, W. H., J. Virol. **15,** 1284 (1975).
2. Chen, M. S., Ward, D. C., and Prusoff, W. H., J. Biol. Chem. **251,** 4833 (1976).
3. Kit, S., and Dubbs, D. R., Biochem. Biophys. Res. Commun. **11,** 55 (1963).
4. Albert, D. M., Lahav, M., Bhatt, P. N., Reid, T. W., Ward, R. E., Cykiert, R. C., Lin, T. S., Ward, D. C., and Prusoff, W. H., Invest. Ophthalmol. **15,** 470 (1976).
5. Lin, T. S., Neenan, J. P., Cheng, Y. C., Prusoff, W. H., and Ward, D. C., J. Med. Chem. **19,** 495 (1976).
6. Dawson, C. R., and Togni, B., Survey Ophthalmol. **21,** 148 (1976).
7. Valenton, M. J., Amer. J. Trop. Med. Hyg., in press (1977).
8. Prusoff, W. H., and Goz, B., Fed. Proc. **32,** 1679 (1973).
9. Calabresi, P., Ardoso, S. S., Finch, S. C., Klingerman, M. M., von Essen, C. F., Chu, M. Y., and Welch, A. D., Cancer Res. **21,** 550 (1961).
10. McGill, J., Williams, H., McKinnon, J., Holt-Wilson, A. D., and Jones, B. R., Trans. Ophthalmol. Soc. U.K. **94,** 542 (1974).
11. Payrau, P., and Dohlman, C., Amer. J. Ophthalmol. **57,** 999 (1964).
12. Coleman, V. R., Tsu, E., and Jawetz, E., Proc. Soc. Exp. Biol. Med. **129,** 761 (1968).
13. Hyndiuk, R. A., Hull, D., Schultz, R., Ching, G., Laibson, P., and Kratcher, J., Amer. J. Ophthalmol. **79,** 655 (1973).
14. Itoi, M., Gefter, J. W., Keneko, N., Ishii, Y., Ramer, R. M., and Gassett, A. R., Arch. Ophthalmol. **93,** 46 (1975).
15. Pavan-Langston, D., Amer. J. Ophthalmol. **80,** 405 (1975).
16. Kurtz, S., *in* "Adenine Arabinoside: An Antiviral Agent", (D. Pavan-Langston, R. Buchanan, and C. Alford, eds.), pp. 145. Raven Press, New York (1975).
17. Nichols, W. W., Cancer Res. **24,** 1502 (1964).
18. Wilkerson, S., Finley, S. C., Finley, W. H., and Ch'ien, L. T., Clin. Res. **21,** 52 (1973).
19. Wellings, P., Awdry, R., Bors, F., Jones, B., Brown, D., and Kaufman, H. E., Amer. J. Ophthalmol. **73,** 932 (1972).
20. Percy, D. H., Albert, D. M., and Amemiya, T., Proc. Soc. Exp. Biol. Med. **142,** 1272 (1973).

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