Activity of 1-β-D-Arabinofuranosylcytosine Hydrochloride Against Herpes Simplex Keratitis. (27884)

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Kaufman(1) recently reported the dramatic activity of 5-iodo-2′-deoxyuridine (IUDR) in treating herpes simplex keratitis in rabbits. Welch and Prusoff(2) had previously described the antitumor properties of this compound. Evans, *et al.*,(3) reported that another pyrimidine nucleoside, 1-β-D-arabinofuranosylcytosine hydrochloride (cytosine arabinoside or CA), also showed antitumor activity and Renis and Johnson(4) reported that CA showed antivaccinia activity. When CA was subsequently found to show marked activity against herpes simplex virus in cell cultures, it became of interest to determine whether this antiherpetic activity could be demonstrated in experimental eye infections. The comparative antiviral activity of CA and of IUDR in treating herpes simplex keratitis in rabbits is described here.

Materials and methods. The MRS strain of herpes simplex virus used in these studies was originally isolated from a herpetic lip lesion. At the time of these experiments, the virus had been through 7 serial passages in rabbit kidney cell cultures and had a titer of approximately 10^{-8}. It was stored in 1-ml aliquots at −70°C in Medium 199 containing 5% calf serum. The identity of the virus was verified by neutralization with herpes simplex immune serum.

Albino rabbits weighing approximately 2 kg each were anesthetized by intravenous injection of 65 mg of sodium pentobarbital. The eyes were infected in one of two ways: in early experiments, the cornea was scratched lightly with the bent tip of a 20-gauge needle and then treated with one drop of the undiluted virus suspension which was spread by closing the lids and rubbing them over the surface of the cornea. Subsequently, it was found that a satisfactory infection could be established by simply rubbing a small sterile cotton swab, dipped into the virus suspension, on the non-scarified cornea of the anesthetized rabbit. Again, this was followed by rubbing the closed lids over the cornea.

To study the effect of test materials on the virus infection, a scoring system based on the appearance of the eye was used:

Score	*Description of the eye*
—	Normal appearance
±	Possible slight infection (minimal conjunctivitis; often possible to detect small punctate lesions with fluorescein staining).
+	Definite infection (marked conjunctivitis accompanied by keratitis; dendritic lesions of the cornea demonstrable with fluorescein).
++	Advanced infection (severe conjunctivitis with purulent exudate initially; spreading dendritic lesions; frequent stromal involvement; cornea insensitive to touch).

Treatment to be administered to a particular group of rabbits was determined before infection. All groups of animals and all chemicals were under code, so that neither the person treating the eyes nor the one doing the scoring was aware of the treatment being given. In addition, each day's scores were made without reference to those recorded on preceding days, to eliminate any tendency to make the scores on a given eye conform to a consistent pattern.

Chemicals to be tested were administered either as drops or in an ophthalmic ointment base, which consisted of a mixture of wool fat, white petrolatum and liquid petrolatum. In the limited testing done with drops, CA was prepared as a 1% solution in physiological saline, IUDR as a 1% solution in 0.03 *N* NaOH. In preparing the corresponding concentrations in ointment, 100 mg of CA was dissolved in 0.2 ml of saline, then mixed thoroughly with 9.8 g of the ointment base; 100 mg of IUDR was dissolved in 1 ml of 0.3 *N* NaOH, then mixed with 9.0 g of the ointment base. The ointment preparations were dispensed from metal tubes at a level of approximately 50 mg/treatment. When

using solutions, 1 drop per treatment was given. In each case, this would represent about 0.25 mg/kg/treatment when using a 1% preparation of the chemical in 2 kg rabbits.

The IUDR was obtained commercially from Cyclo Chemical Corp. The CA was synthesized in the Research Laboratories of The Upjohn Co.

Results. Except for scratches made at time of infection, untreated eyes appeared normal for the first 2 days after infection. However, by the third day they usually showed a marked conjunctivitis, a slightly cloudy cornea and a small amount of exudate. In the typical untreated eye, these early signs of infection progressed rapidly during the following 2 or 3 days. The cornea assumed a milky appearance, the conjunctiva was highly inflamed and the exudate increased in amount. By this time, or within another day or two, the cornea became completely insensitive to touch and there was no apparent vision in the eye. In about one-third of the untreated eyes the infection showed signs of spontaneous regression within 2 weeks after infection, and after an additional week or 2 the eyes appeared normal. In the others, there was residual corneal opacity at least up to a month after infection.

In the first experiment in which the eyes were treated, the compounds were administered as drops. Only the right eye was infected, but treatment was given to both eyes, the left eye serving as a toxicity control. There were 4 rabbits in each group. Treatment was initiated 18 hours after infection by scarification. The control group received saline treatment. All eyes were treated for a total of 150 consecutive hours. Both IUDR and CA showed marked activity in suppressing the infection in this preliminary experiment. Since there was no evidence of drug toxicity, both eyes of each rabbit were infected and treated in subsequent experiments. In addition, test chemicals were prepared in the ophthalmic ointment base rather than in solution, in the hope that it would be possible to obtain significant activity with less frequent treatment.

Several tests were run with these chemicals, in which frequency of treatment, time at which treatment was initiated, length of treatment time and concentration of the chemicals were varied. Two representative experiments will be described in some detail.

The eyes of 15 rabbits were infected by dropping herpes virus on the scarified cornea (day 1). Seventeen hours later treatment was started. Eyes of 6 of the rabbits received the ophthalmic ointment base containing 1% CA; eyes of 4 rabbits received the ointment containing 1% IUDR; eyes of the other 5 rabbits received ointment base only. Each eye was treated hourly from 8:00 a.m. to 5:00 p.m. and every 4 hours at night on days 2, 3 and 4. Only the hourly daytime treatments were given on days 5, 6 and 7. Treatment was then terminated in all eyes except CA #9, 10, 11, 12 and IUDR #1, 2, 3 and 4; these continued to receive hourly treatment on days 8, 9, 10 and 11. The results (Table I) indicate that both CA and IUDR showed outstanding antiherpetic activity in the rabbit eye when treatment was started on the day following infection. Most of the treated eyes which did show infection appeared to have a much milder response than did the virus control eyes. One of the control rabbits died on the tenth day, apparently due to a viral encephalitis.

In another experiment, the eyes of 12 rabbits were infected by rubbing the corneas with a cotton swab saturated with the virus inoculum. Treatment was not started until 40 hours after infection. The eyes of 4 rabbits were treated with 1% CA in the ointment base, eyes of 4 rabbits with 1% IUDR in ointment and eyes of the other 4 with the ointment base alone; in the IUDR-treated group, one eye appeared abnormal at time of infection and was eliminated from consideration, leaving 7 eyes in this group. Each eye was treated hourly from 8:00 a.m. to 5:00 p.m. on days 3, 4, 5, 6, 7 and 8. Scores recorded on these eyes (Table II) indicate that a high level of antiviral activity was obtained with either CA or IUDR, even when initiation of treatment was delayed until 2 days after infection. One of the IUDR-treated rabbits died on the 15th day from an apparent encephalitis; at time of death it

TABLE I. Treatment of Herpes Simplex Keratitis in Rabbits with CA and IUDR, Starting 17 Hours Postinfection. On day 1, all eyes were infected on the scarified cornea. Treatment was given to all eyes on days 2 through 7; in addition, 8 eyes (indicated by *) were further treated on days 8 through 11.

Treatment	Eye No.	Day 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
CA (1%)	1	—	—	—	—	—	—	—	—	—	—	—		+	+	+	+
	2	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—
	3	—	—	—	—	±	—	—	—	—	—	±		±	+	+	++
	4	—	—	—	±	±	—	—	—	—	—	+		++	++	++	++
	5	—	—	—	+	±	±	—	—	±	±	+		+	+	+	+
	6	—	—	—	—	—	—	+	+	+	+	±		+	+	+	+
	7	—	—	—	—	—	—	—	—	—	±	±		+	+	+	+
	8	—	—	—	—	—	—	—	—	—	—	±		±	±	±	±
	9*	—	—	—	—	—	—	—	—	—	—	±		±	+	+	±
	10*	—	—	—	—	—	—	—	—	—	—	—		—	±	±	±
	11*	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—
	12*	—	—	—	—	—	—	—	—	—	—	—		—	±	±	±
IUDR (1%)	1*	—	—	—	—	—	—	—	—	—	±	±		±	±	±	±
	2*	—	—	—	—	—	—	—	—	—	—	—		—	—	—	—
	3*	—	—	—	—	—	—	—	—	—	—	±		±	—	—	—
	4*	—	—	—	+	±	—	—	—	±	±	±		+	++	++	++
	5	—	—	—	—	—	—	—	—	—	—	+		+	+	+	+
	6	—	—	—	—	±	+	+	++	+	++	+		+	+	+	+
	7	—	—	—	—	—	—	—	—	—	—	—		—	—	±	±
	8	—	—	—	—	—	±	±	±	±	+	+		±	+	+	+
Control	1	—	—	—	+	+	++	++	++	++	++	++		++	++	++	++
	2	—	—	—	+	+	++	++	++	++	++	++		++	++	++	++
	3	—	—	—	+	+	++	++	++	++	—Dead—						
	4	—	—	—	+	+	++	++	++	++	"						
	5	—	—	—	±	±	++	++	++	++	++	±		+	+	+	+
	6	—	—	—	±	+	++	++	++	++	++	+		+	+	+	+
	7	—	—	—	+	+	++	++	++	++	++	++		+	+	+	+
	8	—	—	—	+	+	++	++	++	++	++	++		++	++	++	++
	9	—	—	—	±	+	++	+	++	++	++	+		+	±	±	±
	10	—	—	—	+	+	++	++	++	++	++	++		++	++	+	+

Scoring system: — = normal appearance; ± = possible slight infection; + = definite infection; ++ = advanced infection.

showed a 2+ reading in the single eye which was being scored. In both the CA and IUDR groups there was a tendency for the infection to recur after treatment was stopped; however, this infection was generally less severe than that seen in the controls and most of the eyes that had received previous treatment tended to clear spontaneously within a few days when infection did appear.

Discussion. These experiments have demonstrated the efficacy of 1-β-D-arabinofuranosylcytosine hydrochloride in treatment of herpes simplex keratitis in rabbits. They have also confirmed Kaufman's report(1) on the activity of 5-iodo-2′-deoxyuridine in this test system. CA was found to be at least as effective as IUDR in treating the herpes-infected rabbit eye. Published reports indicate that IUDR interferes with incorporation of thymidine into DNA(2) while CA competitively blocks the synthesis of deoxycytidine(4) in addition to causing other metabolic changes(6). In preliminary experiments a combination of IUDR and CA appeared to be somewhat more efficacious than either compound alone. This may indicate a synergistic effect due to the 2 agents interfering with virus synthesis at different points.

The level of activity found with IUDR in these experiments was not quite as dramatic as that initially reported by Kaufman(1); he obtained 100% cures in all rabbits treated, with no evidence of recurrence of the infection after treatment was stopped. In our experiments, more eye to eye variation was found in both treated and virus control groups, and several instances were encountered in which the infection reappeared after treatment was stopped. These differences were probably due to the different strains of

TABLE II. Treatment of Herpes Simplex Keratitis in Rabbits with CA and IUDR, Starting 40 Hours Postinfection. On day 1, all eyes were infected by rubbing the cornea with a virus-saturated cotton swab. Treatment was given to all eyes on days 3 through 8.

Treatment	Eye No.	Day 1	2	3	4	5	6	7	8	9	11	12	13	15	17	19	22
CA (1%)	1	—	—	—	—	—	—	—	±	—	±	+	+	+	—	—	—
	2	—	—	—	—	—	—	—	—	—	±	+	+	++	±	±	—
	3	—	—	—	—	—	—	—	—	—	—	±	±	±	—	—	—
	4	—	—	—	—	—	—	±	±	—	±	+	+	±	±	±	—
	5	—	—	—	—	—	—	—	+	±	+	±	±	±	—	—	—
	6	—	—	—	—	—	—	—	—	—	±	+	±	—	±	—	—
	7	—	—	—	—	±	±	±	+	—	—	±	+	+	—	±	—
	8	—	—	—	—	±	—	—	±	—	±	+	±	—	+	±	±
IUDR (1%)	1	—	—	—	—	±	—	+	+	+	—	±	—	—	—	—	—
	2	—	—	—	—	—	—	—	±	+	±	±	±	—	—	—	—
	3	—	—	—	—	—	—	—	—	—	±	±	++	–Dead–			
	4	Not done												"			
	5	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	6	—	—	—	—	—	—	—	—	—	—	±	±	—	—	±	+
	7	—	—	—	—	—	—	—	+	++	++	++	+	+	+	±	±
	8	—	—	—	—	—	—	—	—	—	—	±	+	—	—	±	±
Control	1	—	—	—	—	+	+	++	++	++	++	++	++	++	++	++	++
	2	—	—	—	—	+	+	++	++	++	++	++	++	++	++	++	+
	3	—	—	±	±	+	+	++	++	++	++	++	++	++	++	++	++
	4	—	—	—	—	±	+	+	++	++	++	++	+	+	±	±	±
	5	—	—	±	±	+	+	++	++	++	+	±	++	++	++	++	++
	6	—	—	—	—	±	±	+	±	—	±	±	±	±	±	±	±
	7	—	—	+	+	+	++	++	++	++	++	++	++	++	++	++	++
	8	—	—	—	—	±	—	+	±	+	±	±	±	±	±	—	—

Scoring system: — = normal appearance; ± = possible slight infection; + = definite infection; ++ = advanced infection.

virus used. As Kaufman later indicated(5), less dramatic cures were obtained when IUDR was tested against herpes strains which caused "stromal" disease. The MRS strain used in our experiments produced severe stromal disease in a high proportion of the control eyes, as well as occasional development of encephalitis and death in these animals. It would appear that this strain was intermediate in severity between the Virtue strain and the highly encephalitic strains. The level of activity reported above for IUDR would appear to conform with that found by Kaufman, when allowance is made for the varying pathogenicity of the virus strains used.

A further factor of importance is the different treatment regimens used. Kaufman (5) emphasized the necessity of treating every 2 hours around the clock. At the time his paper appeared, we had ascertained that it was possible to obtain high levels of activity with intensive treatment during the daytime only, at least when the IUDR was administered in an ointment base. Because of the convenience of this regimen it was continued although it would be anticipated that even higher levels of activity would result from around the clock treatment.

The remarkable antiviral activity of these compounds in treating an established animal virus infection would seem to justify some optimism in the field of viral chemotherapy in general. It is true that the herpes simplex infection on the cornea represents an easily accessible site for drug therapy. However, this may simply mean that one of the major obstacles in successful treatment of other virus infections is one of transport, *i.e.*, having enough of the potential antiviral agent at the infected site for an adequate length of time. It is also encouraging that treatment can be delayed until definite signs of infection are present and a cure can still be effected. It is doubtful that any of the cells already infected with virus are cured; however, it would appear that if spread of the infection to surrounding cells can be stopped, replacement of infected cells with new cells will occur and a cure will be ac-

complished. Thus, the frequently-voiced suggestion that it is already too late to initiate therapy when signs of disease are present in a virus-infected animal would appear to have been overcome, at least in this system.

Summary. The antiviral activity of 5-iodo-2′-deoxyuridine in treating herpes simplex keratitis in rabbits was confirmed. Another pyrimidine nucleoside, 1-β-D-arabinofuranosylcytosine hydrochloride, was found to be at least as effective as IUDR in treating this infection.

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Biochemical Identification of the Carrier State in Tay-Sachs' Disease.* (27885)

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Abnormally elevated levels of various glycolytic, dehydrogenating and transaminating enzymes have been previously demonstrated in sera and spinal fluids of infants with Tay-Sachs' disease (TSD)(1,2). These elevated concentrations, however, were regarded as reflections of the secondary tissue degenerations inherent to the disorder and were not considered to be an indication of any fundamental metabolic defect. Since the disease has been clearly proven to be a hereditary disorder transmitted through an abnormal autosomal recessive gene, parallel enzyme determinations were also performed upon a group of parents of the afflicted children. No analogous serum enzymatic changes were noted in the parents, although it was observed that levels of fructose-1,6-diphosphate aldolase (F-1,6-DPA) were frequently in the low range of normal. These mildly depressed values suggested the need for more specific analysis of serum aldolase and prompted the current study of a closely related enzyme, fructose-1-phosphate aldolase (F-1-PA).

Fructose-1,6-diphosphate is used as the substrate in determination of serum aldolase. It has been shown that F-1-PA may also catalyze the breakdown of this substrate, but at a diminished velocity(3,4). What is conventionally referred to as F-1,6-DPA concentration therefore is a measurement of both F-1,6-DPA and F-1-PA activity, predominantly the former(5). When fructose-1-phosphate serves as the substrate, only F-1-PA produces cleavage to trioses(5). A postulated absence of circulating F-1-PA might result in a small reduction of serum aldolase (fructose-1,6-diphosphate substrate) but would be unmasked only in a system utilizing fructose-1-phosphate. While F-1-PA is sometimes designated as "liver" aldolase, it is nevertheless present in some measure within all normal tissues and serum(6).

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