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Effect of 1-β-D-Ribofuranosyl-1,2,4-Triazole-3-Carboxamide (Virazole, ICN 1229) on Herpes and Vaccinia Keratitis and Encephalitis in Laboratory Animals

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Topical application of 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole) significantly inhibited the development of herpetic keratitis in the eyes of rabbits, as determined by both infectivity and Draize scoring parameters. Significant inhibition of the infection was demonstrated with 10% concentrations of Virazole; a 1% solution had a moderate effect, whereas doses of 0.1 and 0.01% had little activity in this system. A 5% concentration of Virazole similarly inhibited vaccinia keratitis in rabbits. Encephalitis-induced mortality in hamsters initially infected intraocularly with herpesvirus was significantly prevented or inhibited by topical application of 5, 10, and 20% concentrations of Virazole. Surviving, treated hamsters had no signs of herpes keratitis. The 20% concentration was the approximate LD_{50} in hamsters. Virazole administered subcutaneously or intraperitoneally to mice did not appreciably alter the course of herpes virus- or vaccinia virus-induced encephalitis in these animals, although in a herpesvirus experiment direct injection of the drug into the brains 3 hr prior to virus inoculation resulted in a significant survivor increase.

The synthetic triazole nucleoside 1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide (Virazole, ICN 1229) has been shown to have significant activity against members of the herpesvirus and poxvirus families in cell culture systems (4, 6). It is not uncommon, however, for a chemical to exert an antiviral effect in vitro but to have little, if any, activity against the virus in animal systems. Since the in vitro data suggest this compound to have great potential as an antiviral drug, it was important to determine whether the nucleoside would inhibit in vivo virus infections. Experiments performed to demonstrate this in vivo efficacy against type 1 herpes simplex virus (HSV/1) and against vaccinia virus (VV) are described in this report.

MATERIALS AND METHODS

Drug preparation. The Virazole used in these studies was synthesized at this Institute according to the method described by Witkowski et al. (8). The white crystalline chemical was dissolved in 1.4% polyvinyl alcohol (PVA) or in a Jellene base ophthalmic ointment containing 1% chloramphenicol (Parke, Davis & Co., Detroit, Mich.) for topical application; it was dissolved in sterile physiological saline for injection.

Viruses. The McKrae strain of HSV/1, obtained from A. B. Nesburne of the Estelle Doheny Eye Foundation (Los Angeles, Calif.), was used in the rabbit experiments. Strain 123 (7) of HSV/1 was employed in the hamster and mouse experiments. Both viruses were originally isolated from patients with herpes keratitis. The WR strain of VV used in these studies was obtained from Frank M. Schabel, Jr., of Southern Research Institute (Birmingham, Ala.).

Animals. New Zealand albino female rabbits (Mission Laboratory Supply Company, Los Angeles, Calif.) weighing 3 to 5 kg were used in the keratitis experiments. Syrian golden female hamsters (Lakeview Hamster Colony, Newfield, N.J.) weighing 45 to 55 g were used in the herpes keratitis-encephalitis studies, and female Swiss mice (Horton Animal Supply Company, Oakland, Calif.) weighing 18 to 21 g were utilized in the encephalitis experiments.

Rabbit herpetic keratitis experiment. Both eyes in each rabbit were anesthetized with two drops of 0.5% proparacaine HCl (Allergan Pharmaceuticals, Irvine, Calif.). The corneal epithelium was then uniformly scratched three times horizontally and vertically with an inoculating needle, and two drops of a suspension containing approximately 10 ED_{50} of HSV/1 were added to each eye. The eyelids were held closed and slightly

massaged for 1 min. In the first experiment, 10% Virazole in PVA was administered to one eye in each of five rabbits hourly from 8 AM to 7 PM daily for 7 days, with an 8 PM treatment daily of the drug in ointment. Treatment began 4 hr after virus inoculation. The remaining eye in each rabbit was similarly treated with PVA and ointment devoid of drug to serve as a control. The treated eye alternated with each rabbit to provide a more uniformly blind basis for grading. Each eye was examined daily, both grossly and, after fluorescein staining, by biomicroscope, for infectivity (lesion size and type, corneal opacity) and for Draize response (erythema, chemosis, discharge), with the use of a modification of the weighted grading scale described by Corwin et al. (3). In this method, scores of 0 (uninfected) to 4 (maximal severity) were assigned to each infectivity reading, and the daily cumulative infectivity scores were multiplied by 10. Similar scores were assigned for each Draize parameter, and the daily cumulative Draize scores were multiplied by 2. The combined, weighted infectivity and Draize scores of the control and drug-treated eyes were then plotted together and compared.

Rabbits whose eyes were scratched but not exposed to virus were similarly treated with Virazole in parallel in each experiment as toxicity controls.

In a second experiment, the effects of dosages of 1, 0.1, and 0.01% Virazole on herpetic keratitis were compared. In this experiment, both eyes of four rabbits received treatment with each drug dosage and, similarly, both eyes of four virus-control rabbits were treated with placebo. The manner of grading and scoring was the same as in the first experiment.

Rabbit vaccinia keratitis experiment. The vaccinia keratitis experiment was run in a similar manner to the second herpetic keratitis experiment described above, except that treatment began 24 hr after virus inoculation and continued for 9 days. A 5% concentration of Virazole was used.

Hamster keratitis-encephalitis experiments. Hamster eyes were infected with HSV/1 by corneal scarification according to the detailed procedure described in a previous report (7). Animals infected in such a manner develop an opaque area on the cornea as early as 24 hr after the inoculation, which progresses to dendritic patterns associated with herpes keratitis. Approximately 4 days after inoculation, the animals develop signs of central nervous system involvement, and death from encephalitis and meningitis ensues approximately 4 days later. In this chemotherapy experiment, Virazole dissolved in ointment was administered to both eyes twice daily for 15 days beginning 2 hr after virus inoculation. Virus control hamsters were concomitantly treated with ointment devoid of drug. The animals were observed for signs of disease, and deaths were recorded daily for 21 days. The eyes of surviving animals were fluorescein-stained and examined by biomicroscope for evidence of keratitis. Toxicity control hamsters were sham-infected and treated similarly with each drug.

Mouse encephalitis experiments. An initial experiment was performed wherein 15 or 7.5 mg/kg of Virazole dissolved in saline was injected directly into the brains of mice 3 hr pre-, 3 hr post-, or 6 hr post-intracerebral (ic) HSV/1 (10 LD_{50}) inoculation. A detailed account of this target organ treatment methodology was recently described by Allen and Sidwell (1). Ten mice were used in each treatment group. In a second series of experiments, animals were injected ic with 10 LD_{50} of HSV/1 or VV. Virazole, in daily dosages of 1,000, 100, or 10 mg/kg, was administered subcutaneously (sc) four times daily for 7 days, beginning 24 hr before virus inoculation. In a third study, mice were again inoculated ic with HSV/1 or VV and were treated with 100 or 50 mg of Virazole per kg per day administered intraperitoneally (ip) twice daily for 9 days beginning 4 hr before virus inoculation. In a final experiment, mice were inoculated ip with herpesvirus and treated as described above in the third study. In all experiments, virus control mice were treated with the placebo (sterile saline) concomitantly with the test animals. Antiviral activity was evaluated by increases in mean survival time or in survivor number among the treated, infected mice (5).

Toxicity control mice, which were sham-infected, were also included in each test. The animals were examined for 21 days, and deaths occurring were recorded daily.

RESULTS

An initial herpetic keratitis experiment in rabbits which was carried out with 10% Virazole is summarized in Fig. 1. The treated eyes showed a significant improvement over the control eyes by all scoring parameters, although by day 7 small dendrites were observed on the treated eyes. By this time, the dendrites had progressed to geographic ulcers covering the majority of the cornea in the virus control eyes. When the ex-

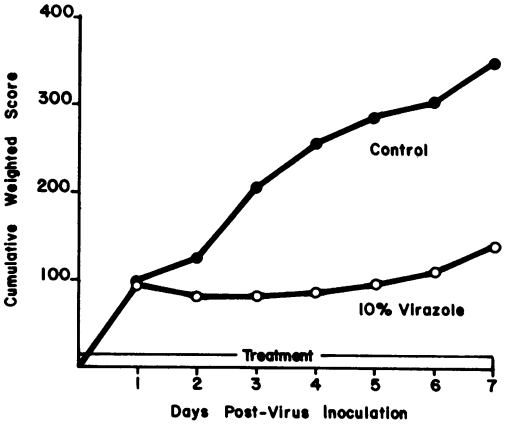

FIG. 1. *Effect of 10% Virazole on herpes simplex keratitis in rabbits.*

periment was repeated with 1, 0.1, and 0.01% Virazole (Fig. 2), inhibition of the virus infection was again manifested, although to a lesser extent than that seen with the 10% concentration. This inhibition was essentially proportional to the concentration of Virazole used. No eye toxicity was noted in any experiment.

Vaccinia keratitis in rabbits was markedly inhibited by treatment with a 5% solution of Virazole (Fig. 3). By day 9 in this experiment, geographic lesions covering virtually the entire cornea were observed in six of eight of the virus control eyes. The remaining two virus control eyes had dendritic lesions covering over 50% (3+) of the cornea. Among the Virazole-treated eyes, one had a geographic lesion covering 50% (2+) of the cornea, and two had dendritic lesions of 2+ and 1+ scores. The remaining five treated eyes had no evidence of infection at this time.

The hamster experiments with 20, 10, and 5% Virazole are summarized in Table 1. After 21 days, 95% of the virus control hamsters which had been treated with ointment devoid of drugs had died, whereas in the group treated with 10% Virazole 40% remained alive, and a significant increase in mean survival time was seen with those treated animals that died during the experiment. The 20% dose of Virazole was somewhat toxic, as evidenced by severe diarrhea and death in two out of five toxicity control animals. None of the infected animals treated with this dose of drug survived the infection, although a moderately significant increase in mean survival time was seen. Treatment with the 5% concentration likewise caused an increase in mean survival time. No signs of herpetic keratitis could be seen on day 21 in any of the infected animals which survived.

Direct Virazole injection into the brains of mice 3 hr prior to HSV/1 inoculation resulted in a significant number of mice surviving the infection (Table 2). This antiviral effect was not seen if the drug was administered 3 to 6 hr after the virus, however, as determined by a lack of survivors and by essentially no increase in mean survival time.

Virazole administered ip or sc into mice infected with herpes encephalitis failed either to prolong the mean survival time of the animals or to increase the number of survivors in any of the experiments carried out. No deaths occurred in any of the toxicity control mice, although on day 7 those treated with daily dosages of 1,000 and 100 mg/kg had an average weight 16% less than normal mice carried concurrently. By day 21, these toxicity control animals weighed 8% less than the normal mice. No other signs of toxicity were manifest during the experiment. Similar treatment of vaccinia encephalitis resulted in a 20% increase in survivor number and an increase in mean survival time of 0.6 days. These increases were not statistically significant.

DISCUSSION

The data presented indicate that the synthetic triazole nucleoside Virazole has significant activity against both HSV/1 and VV topical infections in rabbits. This antikeratitis activity

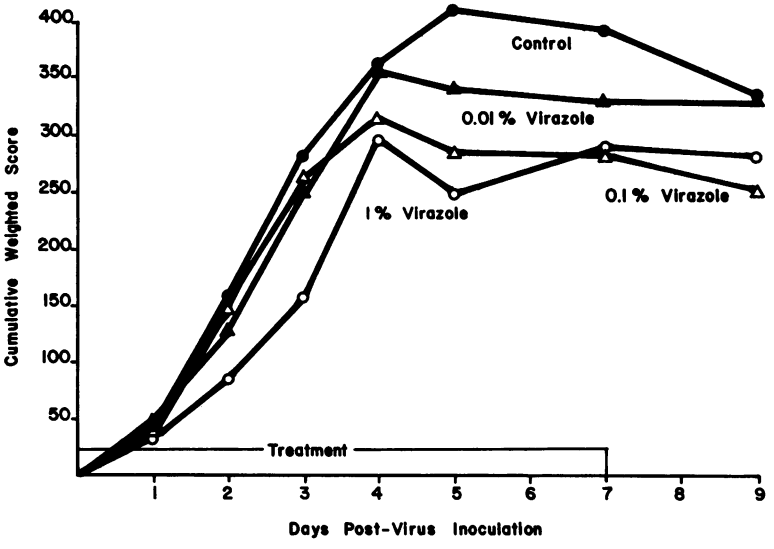

FIG. 2. *Effect of 1, 0.1, and 0.01% Virazole on herpes simplex keratitis in rabbits.*

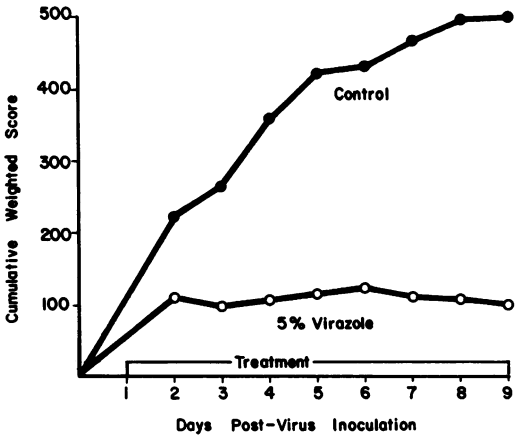

FIG. 3. *Effect of 5% Virazole on vaccinia keratitis in rabbits.*

TABLE 1. *Effect of topically[a] applied Virazole on herpes simplex virus encephalitis-induced deaths in hamsters*

Virazole concn (%)	Treatment group	No. of survivors total	P^b	Mean survival time (days)	P^c
20	Infected animals	0/10	—	9.0	<0.05
	Toxicity control	3/5			
10	Infected animals	4/10	<0.05	10.0	<0.01
	Toxicity control	5/5			
5	Infected animals	0/10	—	8.9	<0.05
	Toxicity control	5/5			
0	Virus control	1/20		7.5	

[a] Jellene base ointment containing drug administered twice daily for 15 days, starting 2 hr after virus inoculation.
[b] Probability (chi-square analysis).
[c] Probability (Student's *t* test).

was equally seen by all scoring parameters; thus, the drug was not only effective in inhibiting the inflammatory (Draize) reaction to the viruses, but it also acted against the development of the virus-induced lesions as determined by scoring of opacity and by biomicroscope examination of fluorescein-stained corneas. The chemotherapeutic index of the drug was relatively low, however, since the activity seen was only at the higher dosages used.

TABLE 2. *Effect of intracerebrally administered Virazole on herpes simplex virus encephalitis-induced deaths in mice*

Time of treatment[a]	Virazole concn (mg/kg)	Survivors/ total	P^b
3 hr pre	15	6/10	<0.01
	7.5	2/10	<0.1
	0	0/10	
3 hr post	15	0/10	—
	7.5	0/10	—
	0	0/10	
6 hr post	15	1/10	>0.3
	7.5	0/10	—
	0	0/10	

[a] Relative to virus inoculation.
[b] Probability (chi-square analysis).

Type 1 HSV infections of the hamster eye generally progress to the central nervous system and the animal dies of encephalitis and meningitis. Since Virazole was applied only topically in the hamster experiments, the increase in survival number and mean survival time of the treated hamsters in this study was, therefore, probably a result of a virus-inhibitory action of the drug in the eye which prevented or at least inhibited the virus in its invasion of the central nervous system. Similar antiviral activity has been seen in earlier hamster studies with iododeoxyuridine (7).

The failure of parenterally administered Virazole to inhibit herpes and vaccinia encephalitis in mice may be attributed to the possible inability of the compound to cross the blood-brain barrier, since it is understood that the ability of drugs to cross that lipoid membrane is a function of the dissociation and the lipid solubility of the nonionized molecules (2). Virazole is not dissociated and is quite lipid-insoluble.

It is significant that, when the drug was injected into the brain prior to the virus, the majority of the mice survived the infection. Arabinosyladenine (ara-A), arabinosylcytosine, and trifluorothymidine also have significant activity in this target organ treatment system, as we have previously reported (1). 5-Iodo-2′-deoxyuridine injected ic in that previous study was not active against the HSV/1 brain infection, although the relative insolubility of that

drug may have been an important factor in this negative result. In that earlier study, the optimal time of treatment with ara-A was 6 hr after virus inoculation, suggesting that Virazole is more active as a prophylactic agent against such infections. Such a conclusion is somewhat contrary to the mode of action seen in vitro (4), wherein the compound could be added up to 24 hr after the virus and still be significantly effective, although the time of in vitro activity seen was dependent to some extent on the multiplicity of infection. Virazole may be exerting its antiviral action in vivo as the 5'-phosphate, as seen in mechanism of action studies (D. G. Streeter et al., *in preparation*); the necessary phorphorylation, therefore, may take sufficient time in the brain that the later times of injection, which were not effective, may consequently have been too late to curb the rapidly progressing and highly lethal viral encephalitis.

The apparent low chemotherapeutic index of Virazole against these deoxyribonucleic acid virus infections, coupled with its apparent inability to cross the blood-brain barrier, suggest limitations to the practical use of the drug. Virazole is readily water-soluble, a property of considerable advantage, particularly for topical application, since it may be able to inhibit deep-seated virus infections more effectively than less soluble materials. The apparent broad-spectrum antiviral effects of the drug are an important additional characteristic.

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