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NOTES

Relative Potencies of Different Anti-Herpes Agents in the Topical Treatment of Cutaneous Herpes Simplex Virus Infection of Athymic Nude Mice

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Thirteen established anti-herpes compounds have been directly compared in a single assay system for their effects on the development of herpetic skin lesions, and mortality associated therewith, in athymic nude (nu/nu) mice inoculated intracutaneously with herpes simplex virus type 1 (KOS). When applied topically (at 1% in a water-soluble ointment), phosphonoacetic acid, *E*-5-(2-bromovinyl)-2'-deoxyuridine, acycloguanosine, and trisodium phosphonoformate emerged as the most active agents.

Quite a variety of compounds have been reported to inhibit selectively herpes simplex virus replication, as recently reviewed by De Clercq (Arch. Int. Physiol. Biochim., in press) and De Clercq and Torrence (5). Use of different procedures for evaluating the in vitro and in vivo potentials of the anti-herpes compounds makes it difficult to rate their relative potencies. We have now evaluated 13 anti-herpes compounds in the same model system, cutaneous herpes simplex virus infection in athymic nude mice (3).

Twenty-five-day-old athymic nude mice (nu/nu), weighing about 16 to 18 g, were inoculated intracutaneously in the lumbar area with herpes simplex virus type 1 strain KOS (approximately 4.7 $\log_{10}$ plaque-forming units per mouse). Vesicles appeared on day 4 postinfection; these vesicles evolved to erosions which ulcerated and progressed downward from the site of inoculation to reach the midline of the abdomen 6 to 8 days postinoculation. After 6 days, control mice started to show symptoms of neurological involvement and paralysis which finally resulted in death. All mice were treated topically (twice daily) for 6 days, starting immediately after virus inoculation, with a water-soluble ointment (Beeler base) containing no active ingredient (control ointment) or 1% of the test compound.

The origins of the test compounds were as follows: phosphonoacetic acid (8), Abbott Laboratories, North Chicago, Ill.; acycloguanosine [9-(2-hydroxyethoxymethyl)guanine] (7), Wellcome Research Laboratories, Research Triangle Park, N.C. (courtesy of G. B. Elion); adenine arabinoside, Parke, Davis Clinical Research Western Europe, Munich (courtesy of R. Wolf); cytosine arabinoside, The Upjohn Co., Puurs, Belgium; thymine arabinoside, Terra-Marine Bioresearch, La Jolla, Calif.; 5-iodo-2'-deoxyuridine, Ludeco, Brussels, Belgium; 5-ethyl- and 5-propyl-2'-deoxyuridine, see reference 5; trisodium phosphonoformate (6), Astra Läkemedel AB, Södertälje, Sweden (courtesy of E. Helgstrand); 5-propynyloxy-2'-deoxyuridine, see reference 10; 5'-amino-5-iodo-2',5'-dideoxyuridine, Calbiochem, La Jolla, Calif.; 5-iodo-2'-deoxycytidine, Serva Feinbiochemica, Heidelberg, West Germany; *E*-(2-bromovinyl)-2'-deoxyuridine, see reference 3.

The results are summarized in Table 1. At 6 days after virus inoculation almost 80% of the control mice had developed herpetic skin lesions, and by day 10 about half of the mice had succumbed to the infection. Treated mice showed a delayed evolution of lesions and a delayed mortality rate, depending on the nature of the compound applied. Cytosine arabinoside and 5'-amino-5-iodo-2',5'-dideoxyuridine did not markedly inhibit the development of lesions (as exemplified by the 80% incidence of epidermal

TABLE 1. *Effects of antiviral compounds on the incidence of herpetic skin lesions and mortality of athymic nude mice inoculated intracutaneously with herpes simplex virus type 1 (KOS)*[a]

Compound	No. of mice with epidermal lesions (necrosis of at least 5–10 mm in length)/total no. of mice alive at day:									Mean survival time (days)
	4	6	8	10	12	14	16	18	20	
Control	0/30	23/30	24/24	13/13	7/7	3/3	2/2	1/1	0	10
Trisodium phosphonoformate	0/10	2/10	1/8	1/8	5/6	4/5	3/4	3/3	1/1	14
Phosphonoacetic acid	0/10	0/10	0/10	1/10	4/10	3/9	2/8	1/7	1/6	>20
5-Iodo-2′-deoxyuridine	0/10	5/10	9/10	5/5	1/1	1/1	1/1	1/1	1/1	10
5-Iodo-2′-deoxycytidine	0/10	3/10	5/10	6/7	4/4	2/2	2/2	1/1	1/1	11.5
5′-Amino-5-iodo-2′,5′-dideoxyuridine	0/10	8/10	9/9	3/3	2/2	1/1	0	0	0	9
5-Ethyl-2′-deoxyuridine	0/10	2/10	9/9	6/6	3/3	0	0	0	0	11
5-Propyl-2′-deoxyuridine	0/10	2/10	7/7	4/4	3/3	1/1	0	0	0	9
5-Propynyloxy-2′-deoxyuridine	0/10	0/10	8/10	4/4	3/3	2/2	2/2	2/2	0	9.5
E-5-(2-bromovinyl)-2′-deoxyuridine	0/10	0/10	0/10	5/10	8/8	7/7	5/5	5/5	2/2	17
Thymine arabinoside	0/10	1/10	6/9	6/7	2/3	3/3	2/2	0	0	11
Cytosine arabinoside	0/10	8/10	9/10	6/6	2/2	0	0	0	0	11
Adenine arabinoside	0/10	3/10	8/10	6/6	6/6	4/4	2/2	1/1	0	13
Acycloguanosine	0/10	0/10	2/10	2/9	5/9	5/7	4/6	2/4	2/3	17

[a] Twenty-five-day-old athymic nude (nu/nu) mice weighing about 16 to 18 g were inoculated intracutaneously in the lumbar area with herpes simplex virus type 1 strain KOS (approximately $10^{4.7}$ plaque-forming units/mouse) and treated topically twice daily for 6 days, starting immediately after virus inoculation, with a water-soluble ointment containing 1% active ingredient.

lesions at day 6 postinoculation). 5-Iodo-2′-deoxyuridine conferred a slight inhibition of vesicle formation (50% of the mice developed lesions by day 6), whereas 5-iodo-2′-deoxycytidine, 5-ethyl-2′-deoxyuridine, 5-propyl-2′-deoxyuridine, thymine arabinoside, and adenine arabinoside offered a somewhat greater protection (20 to 40% of the mice with lesions on day 6). 5-Propynyloxy-2′-deoxyuridine reduced the appearance of herpetic skin lesions at day 6 to 0% but could not prevent the ultimate appearance of these lesions at later times. The most dramatic effects were obtained with trisodium phosphonoformate, acycloguanosine, *E*-5-(2-bromovinyl)-2′-deoxyuridine, and phosphonoacetic acid. These compounds effectively delayed or suppressed, or both, the development of skin lesions and also prolonged the mean survival time to 14, 17, 17, and >20 days, respectively (as compared to 10 days for the control group). Of these four compounds, only phosphonoacetic acid caused a significant increase in the number of survivors (6 of 10, as compared to 0 of 30 for the control group). The fact that in the control group, as in many other groups, the mortality rate reached 100% can probably be ascribed to the high virus dose used for infection. However, this high virus multiplicity appeared necessary for the regular development of skin lesions.

Neither phosphonoacetic acid, trisodium phosphonoformate, nor *E*-5-(2-bromovinyl)-2′-deoxyuridine was found to inhibit epidermal lesions when a 2% ointment application was started at the moment the vesicles had appeared (at day 4) (data not shown). Acycloguanosine was not included in the latter experiment. When applied as a 0.1% ointment for 6 days (starting immediately after virus infection), phosphonoacetic acid and *E*-5-(2-bromovinyl)-2′-deoxyuridine, but not acycloguanosine, inhibited the development of skin lesions (60% as compared to 100% for the control group on day 8; data not shown). With this treatment regimen phosphonoacetic acid increased the mean survival time to 16 days, as compared to 11 days for *E*-5-(2-bromovinyl)-2′-deoxyuridine, 10 days for acycloguanosine, and 10 days for the control group.

At the doses used none of the products caused toxic side effects, except phosphonoacetic acid, which provoked a slight skin irritation in some animals when applied as a 2% ointment.

Our results indicate the following order of activity: phosphonoacetic acid > *E*-5-(2-bromovinyl)-2′-deoxyuridine ∿ acycloguanosine > trisodium phosphonoformate > adenine arabinoside > thymine arabinoside ∿ 5-propynyloxy-2′-deoxyuridine ∿ 5-ethyl-2′-deoxyuridine ∿ 5-propyl-2′-deoxyuridine, 5-iodo-2′-deoxycytidine, 5-iodo-2′-deoxyuridine > cytosine arabinoside ∿ 5′-amino-5-iodo-2′,5′-dideoxyuridine. It should be emphasized that the test compounds were uniformly applied at the same concentration (1%, wt/wt) in the same vehicle (Beeler base ointment). Thus, the relative potencies of the

compounds as recorded herein may or may not accurately reflect the relative efficacies of the compounds when applied at their optimal (maximum tolerated) dosage levels in the most suitable delivery form (vehicle). Obviously, a study of the relative efficacies could only be initiated after the optimal dosage and vehicle would have been determined for each individual compound.

The data obtained herein for phosphonoacetic acid, trisodium phosphonoformate, and acycloguanosine confirm the favorable results observed for these compounds in the topical treatment of cutaneous herpesvirus infection in guinea pigs (1, 2, 7). In our test system, acycloguanosine and *E*-5-(2-bromovinyl)-2′-deoxyuridine yielded better results than the standard anti-herpes compounds 5-iodo-2′-deoxyuridine and adenine arabinoside. *E*-5-(2-bromovinyl)-2′-deoxyuridine and acycloguanosine may therefore be advocated for the local treatment of herpetic skin (or mucosa) lesions. Whether these compounds also affect recurrent herpes infections when applied topically remains to be explored.

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