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Research Article

Efficacy of 5-Vinyl-1-β-D-arabinofuranosyluracil (VaraU) Against Herpes Simplex Virus Type 2 Strains in Cell Cultures and Against Experimental Herpes Encephalitis in Mice: Comparison with Acyclovir and Foscarnet

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The sensitivity of different herpes simplex virus type 2 (HSV-2) strains to inhibition by 5-vinyl-1-β-D-arabinofuranosyluracil (VaraU) was evaluated in comparison to 9-(2-hydroxyethoxymethyl)guanine (ACV; acyclovir) and trisodiumphosphonoformate (Na_3PFA; foscarnet), using a plaque inhibition assay in primary rabbit testes (PRT) cells as well as in human embryonic lung fibroblast (HELF) cell cultures. The order of decreasing activity found was ACV ≫ VaraU > Na_3PFA in PRT cells and ACV > VaraU ≫ Na_3PFA in HELF cells, with 50% inhibition doses (ID_{50}) of 1.8, 8.8, and >110 μ*M* for the three drugs in HELF cells, respectively. After 72hr of drug treatment, inhibition of HELF cell proliferation by VaraU (ID_{50}, >1000 μ*M*) was less than that by ACV and Na_3PFA, resulting in high selectivity indexes of >100 against HSV-2 for VaraU and ACV. Their *in vivo* efficacy was assessed in a mouse encephalitis model. Using a treatment schedule of three daily intraperitoneal (ip) doses over a period of 5 days, only the survival times of mice were considerably prolonged by VaraU (150 or 300 mg/kg per day; $P < 0.05$ or $P < 0.001$, respectively). In contrast, ACV treatment (150 mg/kg per day) led to a nearly complete prevention of encephalitis and death ($P < 0.001$). Similar therapy results with VaraU application through the drinking water were obtained using only one-sixth of the high ip dose (~50 mg/kg per day) but over a prolonged period of treatment. Under similar conditions no therapeutic effect of oral Na_3PFA was observed.

Key Words: 5-vinyl-1-β-D-arabinofuranosyluracil; acyclovir; foscarnet; herpes simplex virus type 2; antiviral activity; experimental herpes encephalitis.

INTRODUCTION

During the last 10 years chemotherapy of herpes virus infections has made great progress (1). The discovery and development of drugs with high potency and selectivity against herpes viruses, i.e., 9-(2-hydroxyethoxymethyl)guanine (acyclovir; ACV) (2) and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (bromovinyldeoxyuridine; BrVUdR; BVDU) (3,4), revealed the possibility of systemic treatment of life-threatening herpesvirus diseases in humans. Whereas in cell culture BrVUdR and ACV inhibit the replication of herpes simplex virus type 1 (HSV-1) at concentrations of nearly 0.02 and 0.2 μ*M*, respectively, ACV exhibits a 10 times and BrVUdR an even 100 to 1000 times lower potential against herpes simplex virus type 2 (HSV-2) (5,6). The failure of topical and systemic BrVUdR treatment in experimental animal HSV-2 infections has been demonstrated (7,8). Intensive efforts in the search for more potent anti-HSV-2 compounds by modifying important features in the structure of BrVUdR (5-X-vinyl substituent, pyrimidine base, sugar moiety) were without success (9–12). A number of potent and selective HSV-1 inhibitors emerged, but the large gap between anti-HSV-1 and anti-HSV-2 activity remained. For example, the exchange of deoxyribose by arabinose resulted in an analogue —(*E*)-5-(2-bromovinyl)-1-β-D-arabinofuranosyluracil (BrVaraU)—which is a highly effective inhibitor of HSV-1 replication in cell cultures and in experimental animal model infections (13–16), but the replication of HSV-2 is affected only at more than 10^4 times the anti-HSV-1 drug concentration. In contrast, the 5-vinyl-araU derivative (VaraU) not only is a strong inhibitor of HSV-1 *in vitro* (14,17) and effective against HSV-1 encephalitis in mice (16), but also inhibits HSV-2 replication at concentrations similar to those of acyclovir (14), the only drug recommended by the Food and Drug Administration (FDA) for the treatment of primary genital herpes.

We have now assessed the antiviral activity of VaraU

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against a number of clinical HSV-2 isolates in two different cell lines as well as the cytostatic effect on the growth of mammalian cells, including a human embryonic cell line, in order to calculate the selectivity of HSV-2 inhibition. Further, the efficacy of VaraU in comparison to acyclovir and trisodiumphosphonoformate (foscarnet) was evaluated in an experimental HSV-2 encephalitis in mice.

MATERIALS AND METHODS

Compounds

VaraU was prepared as described previously (14). ACV was a generous gift of the Burroughs Wellcome Company (Research Triangle Park, N.C.). Na_3PFA was kindly supplied by Dr. Bertram (VEB Arzneimittelwerk, Leipzig, G.D.R.). For plaque assays and cell growth inhibition studies 20 m*M* stocks and dilutions were prepared in phosphate-buffered saline (PBS) and stored at −20°C. In animal experiments fresh solutions of VaraU (5 or 10 mg/ml for ip treatment and 0.67 mg/ml for oral application), of ACV (5 mg/ml for ip and 0.5 mg/ml for oral treatment), and of Na_3PFA (0.67 mg/ml for oral treatment) in sterile saline or water were made separately for each experiment.

Viruses and Plaque Inhibition Assays

The recent clinical isolates, HSV-2 strain 42 (source, herpes progenitalis), strains 55, 81, and 82 (source, herpes integumentalis), and strains 74, 200, and I.H. (source, herpes genitalis), and the laboratory strain HSV-2-US (source, herpes dermatose) were differentiated by quantitative microneutralization with type-specific antiherpes serum from rabbits, by plaque-forming ability in primary chicken embryo cell cultures (18) or, additionally, according to their low sensitivity (ID_{50}, >1 μ*M*) to bromovinyldeoxyuridine. The strain 74 used in animal experiments was further characterized biologically, demonstrating high neurovirulence and liver necrosis in mice after intracerebral (ic) or intravenous (iv) infection, respectively. Details and procedure of plaque inhibition assays in PRT (6,19,20) and in HELF cells (14,16) were described previously. The concentration of compounds inhibiting plaque formation by 50% (ID_{50}) compared to untreated virus control cultures were obtained graphically from the dose–response curves. The ID_{50} values are the means of two plaque assays performed with three concentrations, each within the inhibitory range of the drugs, and with triplicate cultures.

Cells and Cytotoxicity Assays

The preparation of primary rabbit testes (PRT) cells (6,21), the origin of human embryonic lung fibroblast (HELF) cells and of baby hamster kidney (BHK 21/C13) cells, and details of their cultivation and media (4,22) have been described previously. For growth inhibition studies 4 to 6×10^5 cells in 5 ml of Eagle minimal essential medium supplemented with Earle salts and 10% fetal calf serum (heat inactivated at 56°C for 30 min; Institut für Immunpräparate und Nährmedien, Berlin, G.D.R.) and, additionally, 10% tryptose phosphate broth (Oxoid, London) for BHK 21/C13 cells were seeded together with the appropriate substance dilutions into 50-ml culture bottles. After 72 hr the medium was removed, the cultures were harvested using trypsin/versen, and the cell number in the collected suspensions was determined in a Fuchs–Rosenthal chamber using the trypan blue exclusion method. Cell counts of the treated cultures were expressed as the percentages of new cell generations compared with an untreated control culture. The inhibition data were plotted as dose–effect curves (not shown), from which the 50% inhibitory doses (ID_{50}) were obtained. The ID_{50} values are the means of three cytotoxicity assays with three concentrations within the inhibition interval of the compounds (duplicate cultures and duplicate cell counts). The cytostatic effect of a drug (ID_{50}) was expressed as the concentration of a compound that reduced the number of new cell generations in a treated culture by 50% compared to the number of new generations of cells in an untreated control culture.

Experimental HSV-2 Encephalitis in Mice

Groups of SPF-F1 gray female hybrids (ABD2), weighing 20 ± 1 g (Central Institute of Microbiology and Experimental Therapy, Jena, G.D.R.), were infected ic with 1.2 (Expt. 1) or 1.8 (Expt. 2) $\times 10^3$ plaque-forming units (pfu) of HSV-2 strain 74 in 0.1 ml of physiological saline or with 0.1 ml of physiological saline alone in the mock-infected control group. Beginning 2 hr postinfection (pi) groups of 10 mice were treated intraperitoneally (ip) three times daily (every 8 hr) with 0.2 ml of the VaraU (5 or 10 mg/ml) or ACV (5 mg/ml) solution for 5 days. Virus- or mock-infected control mice were treated with 0.2 ml of Hank's salt solution. In the second experiment mice were treated orally with 0.5 mg of ACV or 0.67 mg of VaraU or Na_3PFA per ml of the drinking water for at least 10 days. The mean drinking rates per day per mouse were 3.0, 1.6, and 2.5 ml, corresponding to doses of ca. 75, 50, and 85 mg/kg per day for ACV, VaraU, and Na_3PFA, respectively. The animals were examined twice daily for typical signs of encephalitis. The time of death pi was recorded and mice were considered to be cured if they remained alive up to 28 days pi and if they produced specific antibodies against HSV-2.

Demonstration of Antibody

From all surviving mice heart blood was withdrawn after the 28th day postinfection and antibody was assayed by the fluorescence antibody technique (FAT) as described elsewhere (6,16).

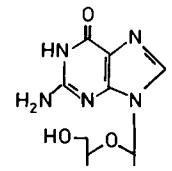
Statistical Analyses

Differences in the efficacy of the nucleoside analogues (Fig. 1) were evaluated with the Gehan test (23,24) on the basis of differences in survival times of mice between the treated groups as well as between the treated groups and the control group. All mice which survived to the end of the test (day 28) were assigned a survival time of 1 day after the test had ended, i.e., day 29.

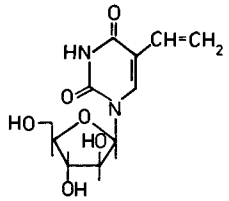
RESULTS

Anti-HSV-2 and Anti-Cell Growth Activity, Selectivity Index

The antiviral activity of VaraU against different HSV-2



9-(2-Hydroxyethoxymethyl) guanine
Acyclovir, ACV, Zovirax®



1-ß-D-Arabinofuranosyl-5-vinyluracil
Vinyl-ara U, Vara U

Fig. 1. Structural formulas of antiherpesvirus nucleoside analogues.

strains, mostly recent clinical isolates, was evaluated in comparison to ACV and Na_3PFA in plaque inhibition assays in PRT and HELF cells. In PRT cells the inhibitory potential decreased in the order ACV ⩾ VaraU > Na_3PFA, with ACV being nearly 80 and 300 times more effective than VaraU and Na_3PFA, respectively (Table I). The inhibition results in HELF cells differed as follows: ACV > VaraU ⩾ Na_3PFA, demonstrating a lower sensitivity of HSV-2 strains to ACV and Na_3PFA but a higher sensitivity to VaraU, which was only four times less active against HSV-2 than ACV (Table II). With VaraU 50% inhibition of HELF and BHK 21/C13 cell proliferation (ID_{50}) was not achieved at concentrations as high as 1000 μM. This was also true of Na_3PFA in BHK 21/C13 cells. In comparison to VaraU, ACV and Na_3PFA exhibited an at least three times stronger cytostatic activity in HELF cells, and ACV even showed an at least eight times stronger inhibition of BHK 21/C13 cell division (Table III). Remarkable indices of selectivity (ID_{50} for cell proliferation divided by ID_{50} for virus plaque formation) were calculated only for ACV and VaraU (~100; Table III).

Treatment of Experimental HSV-2 Encephalitis in Mice

After ic infection with the clinical isolate HSV-2-74 in the first experiment (Fig. 2), all mice in the control group treated with Hank's salt solution died between the 4th and the 12th day (mean, 7.6 days). All mock (saline)-infected and Hank's-treated control mice survived the observation period of 28 days (data not shown). Beginning 2 hr postinfection the treatment of mice by ip injection of VaraU (150 mg/kg per day) over 5 days showed an only marginal prolongation of the survival times (mean, 9.7 days; $P < 0.05$). But treating with a higher VaraU dose (300 mg/kg per day), survival times were considerably prolonged (8–23 days; $P < 0.001$) and one mouse survived (Fig. 2). ACV application (150 mg/kg per day) protected 80% of the HSV-2-infected mice from encephalitis and death ($P < 0.001$).

In the second experiment (Fig. 3) HSV-2 encephalitis of mice was treated orally through the drinking water for 10 days. Untreated infected control mice as well as mice in the Na_3PFA group (84 mg/kg per day) died between the 4th and the 12th day (mean, 6.8 and 6.6 days, respectively). The oral application of VaraU (53 mg/kg per day) gave therapy results similar to those of the high ip dose of 300 mg/kg per day in the first experiment. Survival times were drastically prolonged (5–26 days; $P < 0.001$) and one mouse was still alive at the end of the observation period. Again, oral ACV treatment (74 mg/kg per day) resulted in a strong protection of the mice, with a 70% final survival rate ($P < 0.001$).

The specific antibody response against HSV in the experimentally infected mice was as high in the surviving untreated as in the VaraU-, ACV-, and Na_3PFA-treated animals, respectively. Antibody titers ranged between 1:10 and 1:160.

DISCUSSION

Neonatal herpes, often associated with a diffuse encephalitis process, and primary and recurrent genital herpes with severe complications in immunosuppressed patients are consequences of herpes simplex virus type 2 (HSV-2) infection. The association of both diseases with significant morbidity and mortality has prompted intensive therapeutical trials. Intravenous vidarabine (9-β-D-arabinofuranosyladenine; adenine arabinoside; Ara-A), the first drug recommended for systemic treatment of life-threatening herpesvirus infections, decreases the mortality of neonatal herpes from 70 to nearly 40%, but two-thirds of the surviving babies have severe neurological impairments (25). Low antiviral activity and selectivity in cell cultures, along with low solubility which necessitates a daily 12-hr infusion period, are the main disadvantages of Ara-A. Neurotoxic effects of Ara-A were described (26). The introduction of acyclovir

Table I. Antiviral Activity of VaraU, ACV, and Na_3PFA Against Different HSV-2 Strains in PRT Cells (ID_{50} Values)[a]

Compound	HSV-2 strain						Mean ID_{50} for all strains ± SD
	US	74	55	82	81	200	
ACV	0.34	0.21	0.14	0.2	0.19	0.15	0.21 ± 0.07
VaraU	6.7	25	30	14.1	8	17.4	16.9 ± 9.3
Na_3PFA	>100	51	50	58	70	59	>67.7

[a] Concentration (μM) that reduces the number of plaques by 50% compared with untreated infected control cultures.

Table II. Antiviral Activity of VaraU, ACV, and Na_3PFA Against Different HSV-2 Strains in HELF Cell Cultures (ID_{50} Values)[a]

Compound	HSV-2 strain US	74	55	42	I.H.	Mean ID_{50} for all strains ± SD
ACV	2.4	1.8	1.1	3.4	1.9	2.1 ± 0.86
VaraU	5.9	7.4	13	9	8	8.7 ± 2.67
Na_3PFA	>100	>100	130	—	—	>110

[a] See Table I, footnote *a*.

[9-(2-hydroxyethoxymethyl)guanine; ACV] into clinical trials against neonatal herpes together with vidarabine further decreased the mortality to below 30% (27).

A strong superiority over ACV in the treatment of experimental HSV-2 animal model infections was demonstrated for the new nucleoside analogues, 1-(2'deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC), 1-(2'-deoxy-2'-fluoro-β-D-arabinofuranosyl)-5-methyluracil (FMAU), and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (DHPG) (28–30). However, toxicity in cell cultures has been shown at 1 and 7 μ*M* FMAU and FIAC (31), and both FIAC and DHPG are mutagenic in a sister-chromatid exchange assay at concentrations of 12 and 18 μ*M*, respectively (32). Such serum levels are easily attained by the currently used iv dosage regimens for the systemic treatment of herpesvirus infections in humans.

Our results show that, compared with the anti-HSV-2 nucleoside analogues mentioned above, a considerably lower toxicity can be attested for 5-vinyl-1-β-D-arabinofuranosyluracil (VaraU). The cytostatic effect of potential antiviral compounds on the proliferation of uninfected mammalian cells, especially of human origin, is an important indicator for toxicity in animals and in humans. We found that 50% inhibition (ID_{50}) of baby hamster kidney (BHK 21/C13) and human embryonic lung fibroblast (HELF) cell proliferation was not achieved at VaraU concentrations as high as 1000 μ*M* (25% inhibition), whereas ACV exhibits an at least three to eight times stronger cytostatic effect, i.e., with ID_{50}'s of 130 and 360 μ*M*, respectively (Table III). However, the calculated therapeutic indices (I) in cell culture (ID_{50} for cell proliferation divided by ID_{50} for HSV-2 replication) indicate a high selectivity for both ACV and VaraU (Table III).

Table III. Inhibition of HELF and BHK 21/C13 Cell Growth by VaraU, ACV, and Na_3PFA and Selectivity Indices of the Compounds as Anti-HSV-2 Agents

Compound	ID_{50} (μ*M*) HELF[a]	BHK 21/C13[a]	HSV-2[b]	Selectivity index (I)[c]
ACV	360	130	2.1	62–171
VaraU	>1000	>1000	8.7	>115
Na_3PFA	280	>1000	>110	<2–9

[a] Concentration (μ*M*) that reduces the number of new cell generations in a treated culture by 50% compared to the number of new cell generations in an untreated control culture.

[b] Data from Table II.

[c] I = [ID_{50} (HELF or BHK 21/C13)]/[ID_{50} (HSV-2)].

VaraU lacks mutagenic activity in the AMES (*Salmonella*) mutagenicity test and has only a weak chromosomal abberative potential in Chinese hamster fibroblasts at concentrations of 100 to 500 μ*M*, lower than that of ACV.[5] Only slight differences were observed between antiviral and cytostatic activities of trisodium phosphonoformate (Na_3PFA).

The data presented here indicate that the extent of inhibition of HSV-2 plaque formation by VaraU, ACV, and Na_3PFA was strongly dependent on the host cell line used. In primary rabbit testes (PRT) cells ACV was found to be nearly 80 times more active against HSV-2 strains than VaraU (Table I), but in HELF cells the difference was reduced to the factor 4 (Table II). Comparably, data from earlier investigations had revealed that the anti-HSV-1 potential of VaraU in African green monkey kidney (Vero) cells was 40 to 2000 times weaker than in HELF cells, whereas ACV exhibited comparable inhibition data in both cell lines (14). The same is true for (*E*)-5-(2-bromovinyl)-araU (BrVaraU), a highly effective HSV-1 inhibitor *in vitro* and *in vivo* (13–16) which was practically without anti-HSV-1 activity in Vero cells (14), a cell line used in many laboratories in antiviral screening systems. These data indicate the necessity of evaluating antiviral properties in different host cells, including human cell lines. Comparing the activity spectrum against viruses of the herpes group *in vitro*, further advantages of VaraU over ACV became evident (Table IV). In cell cultures VaraU has a several times stronger inhibitory effect than ACV for different HSV-1 strains and an even 70 to 100 times higher potential against two varicella-zoster virus (VZV) isolates. However, only ACV weakly influenced human cytomegalovirus (HCMV) replication. Both compounds inhibited the expression of Epstein–Barr virus (EBV) antigens in EBV-producing lymphoblastoid (P3HR-1) cells, but only VaraU showed a prolonged inhibitory effect even at 1 μ*M* after release of the drug action, whereas the ACV inhibition was immediately reversible (33).

Our results in the treatment of an HSV-2 encephalitis in mice demonstrate that ACV is superior to VaraU, confirming our findings in cell cultures. Intraperitoneal (ip) VaraU treatment of mice over 5 days did not reduce mortality but did significantly prolong survival times in a dose-dependent manner at both 150 and 300 mg/kg per day (difference significant, with $P < 0.05$). In contrast, ACV treatment (150 mg/kg per day) enhanced both survival time and survival rate (from 0 to 80%), being more effective than the high ip VaraU dose ($P < 0.01$). A therapeutic effect of the compounds tested was also evident from the fact that at the end of the 5-day treatment period, all mice in the drug-treated groups were without any signs of illness, but all placebo-treated infected control mice were ill (data not shown). Apparently, the 5-day VaraU application interval was too short, because after cessation of treatment the mice in the low-dose group and half of the mice in the high-dose group became ill within a mean time of 2.3 and 6.1 days, respectively. A delayed appearance of first disease symptoms and death was recorded for the other animals in the high-dose VaraU group (see Fig. 2), and similar observations were made in the ACV group, where one late death occurred and another two mice became ill at the end of the 28-day obser-

[5] J. Schöneich, unpublished data.

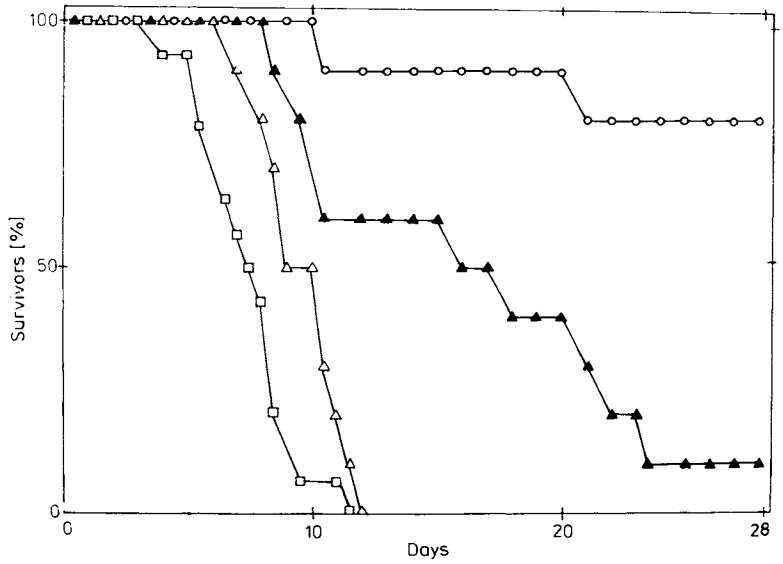


Fig. 2. Comparison of the antiviral potential of VaraU and ACV against intracerebral HSV-2 infection of mice. VaraU, 50 mg/kg (△) or 100 mg/kg (▲), and ACV, 50 mg/kg (○), were administered intraperitoneally 2hr postinfection and, thereafter, three times daily (every 8hr) for 5 days. (□) Infected placebo-treated control.

vation period (data not shown). Whether these late events are related to the early recurrences of HSV-2 infection or to the reversibility of inhibition of virus replication due to low drug levels in the brain remains to be proven.

The results of an oral 10-day drug treatment of HSV-2 encephalitis in mice confirmed the preceding observation that under similar conditions, VaraU is less efficient than ACV. The inability of a comparable oral Na_3PFA dose either to increase the survival rate or to prolong survival times reflects its low anti-HSV-2 potential *in vitro*. Compared with the ip treatment in the first experiment, a similar therapeutic effect of oral drug application was achieved with only one-sixth of the high ip dose and half of the dose of VaraU and ACV, respectively. A comparison of both experiments seems justified because of the same infection procedure, resulting in similar mean survival times of placebo-treated infected control mice, which died within 7.6 ± 1.95 as well as 6.8 ± 2.27 days ($P \geqslant 0.1$). Again, successful therapy by two of the drugs was obvious at the end of the 10-day treatment period regarding the number of mice that died compared

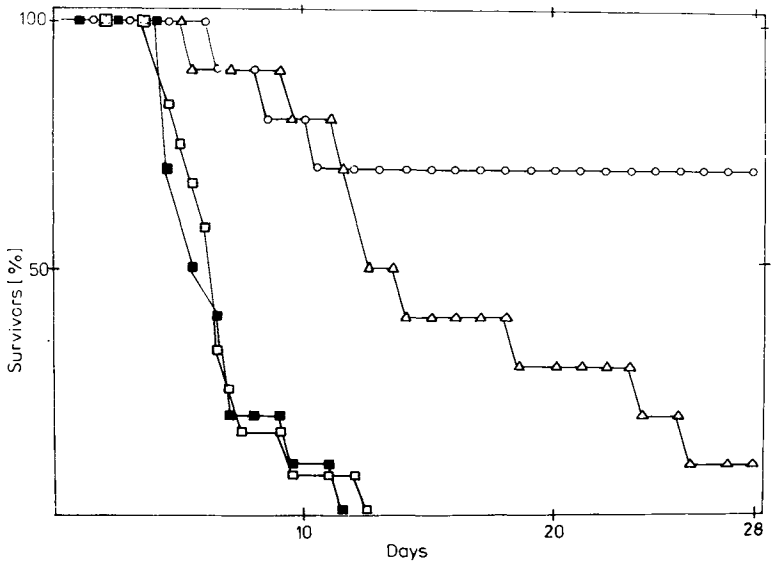


Fig. 3. Comparison of the antiviral potential of VaraU, ACV, and Na_3PFA against intracerebral HSV-2 infection of mice. VaraU, 53 mg/kg per day (△), ACV, 74 mg/kg per day (○), and Na_3PFA, 84 mg/kg per day (■), were administered orally through the drinking water beginning 2 hr postinfection for at least 10 days. (□) Infected placebo-treated control.

Table IV. Antiviral Activities of VaraU and ACV in Cell Cultures

Virus[a]	ID_{50} (μM)[b]	
	VaraU	ACV
HSV-1 (64)[c]	0.042	0.187
HSV-I (511)[c]	0.064	0.120
HSV-1 (K)	0.061	0.139
HSV-2[d]	8.7 ± 2.67	2.1 ± 0.86
VZV (S.H.)[c]	0.084	5.7
VZV (S.G.)[c]	0.125	12.6
HCMV (Davis)	>500	88

[a] The strain is given in parentheses.
[b] Determined by plaque assays in human embryonic lung cells (HELF for HSV-1 and HSV-2; MRC-5 for VZV and HCMV); data for HSV-1 strains from Ref. 16.
[c] Recent clinical isolate.
[d] Mean ID_{50} ± SD for five strains (data from Table II).

with the total number of treated mice, i.e., 11 of 12, 9 of 10, 2 of 10, and 2 of 10 for the control, Na_3PFA, VaraU, and ACV group, respectively. Taking into consideration the different molecular weights of ACV and VaraU, on the one side, the respective comparable oral doses should be 75 and 90 mg/kg per day. On the other side, a four times lower activity of VaraU in cell cultures compared with ACV would imply a comparable total oral VaraU dose of nearly 360 mg/kg per day. However, a more convenient approach for evaluating the *in vivo* efficacy of test compounds consists in comparing 50% effective doses (ID_{50} values) using optimal treatment schedules (30). The oral VaraU dose of nearly 50 mg/kg per day in the present HSV-2 infection is obviously below the dose protecting 50% of the mice from encephalitis and death, whereas the ID_{50} of ACV *in vivo* is less than ~75 mg/kg per day (Fig. 3).

In conclusion, our investigations in the mouse HSV-2 encephalitis model clearly demonstrated the therapeutic value of oral and intraperitoneal VaraU application, although under comparable conditions the therapeutic effect of ACV is superior to that of VaraU. Optimal doses and treatment schedules have yet to be established. The fact that ACV and VaraU are similarly effective in suppressing the extent of liver necrosis in mice after intravenous infection with HSV-2[6] underlines the significance of our HSV-2 encephalitis results. In addition, intraperitoneal VaraU was superior to equimolar ACV in HSV-1 encephalitis of mice (16). If started 3 days after infection, 0.1% VaraU eye drops were effective against experimentally induced HSV-1 keratitis in rabbits,[7] and a 7-day course of treatment, beginning 2hr after infection, reduced the virus titers in the aqueous fluid of the rabbit eye and prevented the establishment of trigeminal ganglion latency (34). Although toxicity studies in uninfected control mice were not done, a lack of toxicity of a 140-mg/kg daily oral VaraU dose given for a period of 6 days for the treatment of HSV-1-infected mice was obvious. Not only were all symptoms of encephalitis and death completely prevented, but mice developed normally without any loss of body weight.[8] The induction of specific HSV antibodies at comparable titers in the experimentally infected untreated as well as in the treated mice indicates a normal immune reaction under VaraU, ACV, and Na_3PFA treatment.

In summary, the present data describe inhibitory properties of VaraU not only against HSV-2 strains in cell cultures but also in the treatment of HSV-2 encephalitis in mice. The low cytostatic activity for a human cell line, the high solubility of the compound (>10 mg/ml at 25°C), and the high efficacy in HSV-1 animal model infections testify to the potential of VaraU as a possible approach to the control of local and systemic herpesvirus infections in humans.

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