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

Jürgen Reefschläger,1,4 Peter Wutzler,2 Klaus-Dieter Thiel,2 and Gottfried Herrmann3

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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 trisodiumphosphonomiformate (Na3PFA; foscarnet), using a plaque inhibition assay in primary rabbit testes (PRT) cells as well as in human embryonic lung fibroblast (HEL) cell cultures. The order of decreasing activity found was ACV > VaraU > Na3PFA in PRT cells and ACV > VaraU > Na3PFA in HEL cells, with 50% inhibition doses (ID50) of 1.8, 8.8, and >110 μM for the three drugs in HELF cells, respectively. After 72 hr of drug treatment, inhibition of HELF cell proliferation by VaraU (ID50 >1000 μM) was less than that by ACV and Na3PFA, 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 Na3PFA 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 μM, 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 (BrVarA)—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 104 times the anti-HSV-1 drug concentration. In contrast, the 5-vinyl-arA 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

1 Institute of Virology, Medical Department (Charité), Humboldt-University of Berlin, DDR-1040 Berlin, Schumannstr. 20-21, G.D.R.
2 Institute of Medical Microbiology, Medical Academy Erfurt, DDR-5010 Erfurt, Nordhäuserstraße 74, G.D.R.
3 Department of Cell Kinetics, Central Institute of Molecular Biology, G.D.R. Academy of Sciences, DDR-1115 Berlin-Buch, G.D.R.
4 To whom correspondence should be addressed.
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 trisodiumphosphonofomitate (toscarnet) 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.). Na₃PFA was kindly supplied by Dr. Bertram (VEB Arzneimittelwerk, Leipzig, G.D.R.). For plaque assays and cell growth inhibition studies 20 mM 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 Na₃PFA (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 genitalis), strains 55, 81, and 82 (source, herpes intregumentalis), 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 antiserum serum from rabbits, by plaque-forming ability in primary chicken embryo cell cultures (18) or, alternatively, according to their low sensitivity (ID₉₀ > 1 μM) 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 HELC cells (14,16) were described previously. The concentration of compounds inhibiting plaque formation by 50% (ID₉₀) compared to untreated virus control cultures were obtained graphically from the dose–response curves. The ID₉₀ 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 × 10⁴ 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 trypsin 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 (ID₉₀) were obtained. The ID₉₀ 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 (ID₉₀) 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 (Exp. 1) or 1.8 (Exp. 2) × 10⁶ 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 Na₃PFA 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 Na₃PFA, 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