

Comparison of Susceptibilities of Varicella-Zoster Virus and Herpes Simplex Viruses to Nucleoside Analogs

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The susceptibilities of varicella-zoster virus (VZV) and herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) to 17 nucleoside analogs were compared by a plaque reduction assay with human embryonic lung fibroblast cells. The susceptibility of VZV to certain nucleoside analogs was different from that of HSV-1. Against VZV the 5-halogenovinyl-arabinosyluracils were the most potent of the compounds tested.

Varicella-zoster virus (VZV) is capable of inducing a virus-specific thymidine kinase (TK) (2, 9) which seems to resemble the herpes simplex virus (HSV)-induced TK in substrate specificity (4). Therefore, one might expect VZV to be susceptible to nucleoside analogs which are active against HSV. Indeed, the *in vitro* inhibition of VZV replication by a number of antiherpes agents has been reported (4, 6, 13). However, these studies did not involve side-by-side assessments of the inhibition of HSV replication. This report involves a direct comparison of the susceptibilities of HSV and VZV to a wide variety of nucleoside analogs. Since cell lines and assay methods can affect the relative susceptibilities of viruses to antiherpes agents (3, 5, 11), we used the same cell culture system and assay method in our comparison. Since both HSV and VZV form clear plaques on human embryonic lung fibroblast (HEL-F) cells, we used these cells and a plaque reduction assay to determine the susceptibilities of HSV type 1 (HSV-1), HSV type 2 (HSV-2), and VZV to 17 nucleoside analogs.

HEL-F cells, strain HAIN-55 (10), kindly supplied by H. Okumura, National Institute of Health of Japan, Tokyo, were used in this study. The level of passage of the cells was 25 to 33 population doublings. Three strains of HSV-1 and three strains of HSV-2 were used as stock strains (7). Three clinical isolates which had been typed as HSV-1 were kindly supplied by T. Kurimura, Tottori University School of Medicine, Yonago, Japan, and by K. Hayashi, Koriyama Institute of Medical Immunology, Koriyama, Japan. Seven strains of VZV (6) and strains CaQu, Batson, and K9 were used. The latter three strains were kindly supplied by S. Shigeta, Fukushima Medical College, Fukushima, Japan, and M. Takayama, National Institute of Health of Japan. These VZV strains were passaged 6 to 20 times in HEL-F cells, except that strains Ohtomo, CaQu, and Batson were passaged more than 100 times and strain K9 was passaged 45 to 48 times. The plaque reduction assay was carried out as described previously (6,7), except that in some experiments the HSV-infected cultures were overlaid with maintenance medium containing 0.8% methylcellulose (3,500 to 5,600 centipoise; Nakarai Chemicals) in place of 0.5% Noble agar (Difco Laboratories) and 50 µg of DEAE-dextran per ml and that if necessary, HSV- and VZV-infected cultures were stained with 1% crystal violet solution. The 50% inhibitory doses (ID₅₀s) of the test compounds for exponentially growing HEL-F cells were determined as described previously (9).

The compounds evaluated included the following: 1-β-D-

arabino-furanosyl-*E*-5-(2-bromovinyl)uracil (BV-araU), 1-β-D-arabino-furanosyl-*E*-5-(2-chlorovinyl)uracil (CV-araU), 1-β-D-arabino-furanosyl-*E*-5-(2-iodovinyl)uracil (IV-araU), and 1-β-D-arabino-furanosyl-*E*-5-(2-bromovinyl)uracil-5'-mono-phosphate (BV-araUMP) (7, 8); 1-β-D-arabino-furanosyl-5-vinyluracil (vinyl-araU), 5-hydroxy-2'-deoxyuridine (OH-dUrd), 5-methoxycarbonylmethyl-2'-deoxyuridine (MCM-dUrd), and 5-acetonyl-2'-deoxyuridine (aceto-dUrd) (12); arabinofuranosylthymine (araT) and 5-iodo-2'-deoxyuridine (I-dUrd) (both from Yamasa Shoyu Co., Ltd.); 1-β-D-arabino-furanosyl-5-ethyluracil (ethyl-araU), *E*-5-(2-bromovinyl)-2'-deoxyuridine (BVDU), and 1-(2-deoxy-2-fluoro-β-D-arabino-furanosyl)-5-vinyluracil (FVAU) (all synthesized by T. Ikeda and T. Yamaguchi, Yamasa Shoyu Co. Ltd., Choshi-shi, Chiba-ken, Japan); 5-ethyl-2'-deoxyuridine (EDU) (kindly supplied by K. K. Gauri, Universität Hamburg, Hamburg, Federal Republic of Germany); 1-(2-deoxy-2-fluoro-β-D-arabino-furanosyl)-5-iodocytosine (FIAC) and 1-(2-deoxy-2-fluoro-β-D-arabino-furanosyl)-5-iodocytosine (FIAC) and 1-(2-deoxy-2-fluoro-β-D-arabino-furanosyl)-5-methyluracil (FMAU) (both kindly supplied by J. J. Fox, Sloan-Kettering Institute, New York, N.Y.); and acyclovir (kindly supplied by W. G. Wilson, Nippon Wellcome Co. Ltd., Shinoo, Osaka, Japan).

Although all of the nucleoside analogs tested (Table 1) were active against the various strains of HSV-1, including the clinical isolates, the 5-halogenovinyl-arabinosyluracils (XV-araUs), BVDU, the 2'-fluoro-arabinosylpyrimidine nucleosides (F-araPyr), and acyclovir were especially active. OH-dUrd, araT, FIAC, FMAU, and acyclovir were almost as active against HSV-2 as against HSV-1. In contrast, HSV-2 was much less susceptible to BVDU, vinyl-araU, XV-araUs, BV-araUMP, ethyl-araU, and aceto-dUrd than was HSV-1. The 50% plaque reduction doses of the latter compounds for HSV-2 were generally two or more orders of magnitude greater than those for HSV-1. HSV-2 strains were, on the average, 10 to 30 times less susceptible than were HSV-1 strains to I-dUrd, MCM-dUrd, and FVAU. Although the compounds varied enormously in potency, there was little variation in the susceptibilities of strains of either HSV-1 or HSV-2, including the clinical isolates, to a particular compound. VZV was more susceptible than HSV-1 to XV-araUs and BV-araUMP and, like HSV-1, was susceptible to the other nucleoside analogs, with the exception of ethyl-araU. However, VZV was less susceptible than HSV-1 to EDU, vinyl-araU, ethyl-araU, aceto-dUrd, and acyclovir. Overall, the variation in the susceptibilities of

TABLE 1. Susceptibilities of HSV-1, HSV-2, and VZV to and inhibition of growth of HEL-F cells by nucleoside analogs

Compound	Amt ($\mu\text{g/ml}$) of test agent required for 50% plaque reduction of:						ID ₅₀ for HEL-F cells ($\mu\text{g/ml}$)	Therapeutic index ^a for:		
	HSV-1 ^b		HSV-2 ^c		VZV ^d			HSV-1	HSV-2	VZV
	Mean	Range	Mean	Range	Mean	Range				
I-dUrd	0.53	0.28–1.00	4.2	1.95–6.2	0.95	0.27–2.45	8	15	2	8
EDU	0.99	0.39–5.6	1.18	1.08–4.7	6.74	1.26–42.0	40	40	34	6
BVDU	0.018	0.008–0.046	9.8	5.8–15.0	0.008	0.0015–0.026	145	8,100	15	18,000
Aceto-dUrd	3.40	1.53–5.4	>1,000	>1,000	18.7	3.2–62	>800	>240		>43
MCM-dUrd	11.2	5.5–42	88.5	26.3–209	2.66	0.40–16.2	400	36	5	150
OH-dUrd	17.5	3.6–32	16.2	14.1–19.2	15.0	2.3–76	>800	>46	>49	>53
AraT	0.16	0.052–0.29	0.42	0.22–0.66	0.063	0.017–0.28	100	630	240	1,600
Ethyl-araU	1.01	0.42–1.85	74	76–261	449	72–995	>800	>790	>11	>2
Vinyl-araU	0.027	0.011–0.071	2.41	1.25–4.0	0.128	0.035–1.0	650	24,000	270	5,100
BV-araU	0.011	0.004–0.030	13.1	7.3–26.3	0.00026	0.00008–0.00063	>800	>73,000	>61	>3,100,000
CV-araU	0.014	0.004–0.025	16.3	8.4–26.6	0.00043	0.00013–0.00145	>800	>57,000	>49	>1,900,000
IV-araU	0.010	0.004–0.024	6.9	4.2–18.0	0.00037	0.00013–0.00071	>800	>80,000	>120	>2,200,000
BV-araUMP	0.011	0.005–0.032	18.3	15.0–26.9	0.00042	0.00023–0.00094	>800	>73,000	>44	>1,900,000
FIAC	0.016	0.008–0.051	0.017	0.011–0.023	0.015	0.0048–0.051	10	630	590	670
FMAU	0.008	0.003–0.038	0.010	0.007–0.013	0.0049	0.0021–0.021	3	380	300	610
FVAU	0.014	0.012–0.020	0.42	0.26–0.55	0.029	0.011–0.060	11	790	26	380
Acyclovir	0.040	0.007–0.14	0.056	0.029–0.11	0.50	0.17–1.78	260	6,500	4,600	520

^a Calculated as the ID₅₀ for HEL-F cells divided by the 50% plaque reduction dose for each virus.

^b Three stock strains and three clinical isolates.

^c Three stock strains.

^d Ten clinical isolates.

different strains of VZV to a particular compound was relatively great.

The inhibition of cell growth by XV-araUs, BV-araUMP, OH-dUrd, aceto-dUrd, and ethyl-araU was less than 50% at a drug concentration as high as 800 $\mu\text{g/ml}$. The antiviral indexes of XV-araUs and BV-araUMP for HSV-1, calculated as the ID₅₀ for HEL-F cells divided by the 50% plaque reduction dose for HSV-1, were greater than 50,000, and those for VZV were greater than 1,900,000. These values were the highest among those of the compounds tested. BVDU, MCM-dUrd, araT, vinyl-araU, and acyclovir also had low inhibitory action on cell growth (ID₅₀s, 100 to 650 $\mu\text{g/ml}$), whereas F-araPyr and I-dUrd were found to have inhibitory action. The antiviral indexes of F-araPyr for HSV were less than 1,000.

The activity of nucleoside analogs against VZV was expected since, like HSV-1 and HSV-2, VZV is capable of inducing virus-specific TK (2, 9). Specific phosphorylation in virus-infected cells is a general phenomenon that has been observed for all selective inhibitors of HSV-1, HSV-2, and VZV. Indeed, all compounds tested in this study, except I-dUrd, have been shown to be selective (7, 12). HSV-induced TK has broad substrate specificity and can phosphorylate many nucleoside analogs (1). If VZV-induced TK has the same substrate specificity and ability to phosphorylate monophosphate nucleotide derivatives as does HSV-1-induced TK, VZV and HSV-1 might be equally susceptible to each compound. However, we found some differences between the susceptibilities of HSV-1 and VZV to the 17 nucleoside analogs tested. (i) VZV and HSV-1 were almost equally susceptible to compounds such as F-araPyr, araT, and 5-substituted deoxyuridines, with the exception of EDU and aceto-dUrd. (ii) VZV was much more susceptible than HSV-1 to XV-araUs and BV-araUMP. (iii) VZV was markedly less susceptible than HSV-1 to the other compounds, including acyclovir, aceto-dUrd, EDU, ethyl-araU, and vinyl-araU. Furthermore, VZV was less susceptible than HSV-2 to ethyl-araU, EDU, and acyclovir, and both VZV and HSV-2 were less susceptible than HSV-1 to nucleoside

analogues having an ethyl or a vinyl residue at the C-5 position of the pyrimidine base.

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