BIKEN JOURNAL Vol. 29, 1–6, 1986

SUSCEPTIBILITY OF VARICELLA-ZOSTER VIRUS TO THYMIDINE ANALOGUES

HARUHIKO MACHIDA

Research Laboratories, Yamasa Shoyu Co., Ltd.
Araoicho 2 chome, Choshi-shi, Chiba, 288, Japan

(Received October 28, 1985)

SUMMARY Ten strains of varicella-zoster virus (VZV) were tested for susceptibility to 17 nucleoside analogues by a plaque reduction assay using human embryonic lung fibroblast cells. The compounds employed were 5-substituted arabinosyluracils and 2′-deoxyuridines, 2′-fluoro-arabinosylpyrimidines (F-araPyr) and acyclovir. In terms of the 50% plaque reduction dose (PD_{50}), 4- to 40-fold difference were found between the 10 strains of VZV in susceptibilities to each compound. VZV was highly susceptible to 5-halogenovinyl-arabinosyluracils (XV-araUs); the PD_{50} values of these compounds were less than 0.001 μg/ml. VZV was much more susceptible than herpes simplex virus (HSV) type 1 to XV-araUs, but less susceptible than either HSV type 1 or type 2 to 5-ethyl-2′-deoxyuridine, 5-ethyl-arabinosyluracil and acyclovir.

INTRODUCTION

Varicella-zoster virus (VZV), like herpes simplex virus (HSV), is capable of inducing virus specific thymidine kinase (TK) (Cheng et al., 1979; Ogino et al., 1977), which seems to resemble HSV-1 induced TK in substrate specificity (De Clercq et al., 1982). Thus VZV was expected to be susceptible to a series of nucleoside analogues that are active against HSV. Indeed, in vitro inhibition of VZV replication by a number of antiherpes agents has been reported (Shigeta et al., 1983), and the susceptibilities of HSV and VZV to some compounds have been compared directly (Crumpacker et al., 1979; Lopez et al., 1980). In these studies, all the compounds with activity against HSV-1 were found to exhibit activity against VZV. We also showed in preliminary studies that anti-herpes virus agents had significant anti-VZV activity (Machida et al., 1982). In this work, I examined the susceptibilities of ten strains of VZV to a wide variety of thymidine analogues including 5-substituted deoxyuridines and arabinosyluracils, 2′-fluoro-arabinosylpyrimidines (F-araPyr) and acyclovir by a plaque reduction assay on human embryonic lung fibroblast (HEL-F) cells, and found that VZV

Mailing address: Dr. H. Machida, Research Lab., Yamasa Shoyu Co., Ltd., Araoicho 2 chome, Choshi-shi, Chiba, 288, Japan

Most of the data were presented at the 6th International Congress of Virology, in Sendai, Japan, on September 1–7, 1985.

was much more susceptible than HSV to 5-halogenovinyl-arabinosyluracils (XV-araUs), but less susceptible than the latter to acyclovir and some thymidine analogues.

MATERIALS AND METHODS

1. *Cells*

HEL-F cells, strain HAIN 55, which were established from human embryonic lung (Okumura et al., 1979) and kindly supplied by Dr. H. Okumura, National Institute of Health of Japan, were used. The methods used for cultivation of the cells were described previously (Machida et al., 1979). The cells were used after 25 to 33 population doublings.

2. *Viruses*

VZV strains YS and Asahikawa were kindly supplied by Dr. T. Sakuma, Asahikawa Medical College, Asahikawa, Japan; strains Oka and Kawaguchi were kindly provided by Dr. M. Takahashi, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan; strains Kanno, Hirai, Ohtomo, CaQu and Batson were kindly supplied by Dr. S. Shigeta, Fukushima Medical College, Fukushima, Japan; and strain K 9 was obtained from Dr. M. Takayama, National Institute of Health of Japan. All these strains of VZV were fresh isolates from patients and were passaged 6 to 20 times in HEL-F cells, except strains Ohtomo, CaQu and Batson (passage level, more than 100), and K 9 (passage level, 45 to 48).

3. *Determination of susceptibility*

A plaque reduction assay was employed for determination of the susceptibility of VZV to drugs. The assay was generally carried out as described previously (Machida et al., 1982), except that the infected cultures were stained with 1% crystal violet solution. The test compound was diluted in serial half-$\log_{10}$ decrements. Confluent monolayers of HEL-F cells obtained 4–5 days after seeding were infected 50 to 100 plaque forming units of VZV. After an adsorption period of 1 hour, the diluted test compound was added to the infected cultures, and incubation was continued for 4–7 days. The number of plaques was counted microscopically and the 50% plaque reduction dose (PD_{50}) for each strain was obtained graphically. Usually, a cell-associated VZV preparation was employed. For assays with strains Oka and Kawaguchi, either a cell-free or cell-associated virus preparation was empolyed, but no differences between the susceptibilities of these preparations were observed.

4. *Compounds*

The following compounds were employed to determine the susceptibility of VZV: 1-β-D-arabinofuranosyl-*E*-5-(2-bromovinyl)uracil (BV-araU), 1-β-D-arabinofuranosyl-*E*-5-(2-chlorovinyl)-uracil (CV-araU), 1-β-D-arabinofuranosyl-*E*-5-(2-iodovinyl)uracil (IV-araU), BV-araU-5′-monophosphate (BV-araUMP) (Machida and Sakata, 1984); 1-β-D-arabinofuranosyl-5-vinyluracil (vinyl-araU), 5-hydroxy-2′-deoxyuridine (OH-dUrd), 5-methoxycarbonylmethyl-2′-deoxyuridine (MCM-dUrd), 5-acetonyl-2′-deoxyuridine (aceto-dUrd) (Sakata et al., 1980); 1-β-D-arabinofuranosyl-5-ethyl-araU (ethyl-araU) (Machida et al., 1979); arabinofuranosylthymine (araT), 5-iodo-2′-deoxyuridine (IDU) (commercial products from Yamasa Shoyu Co.). *E*-5-(2-bromovinyl)-2′-deoxyuridine (BVDU), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-vinyluracil (FVAU) (synthesized by Dr. T. Ikeda and Mr. T. Yamaguchi, Yamasa Shoyu Co.); 5-ethyl-2′-deoxyuridine (EDU), kindly supplied by Dr. K. K. Gauri, Unversität Hamburg, Hamburg; 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC), 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil (FMAU) (Lopez et al., 1980), kindly supplied by Dr. J. J. Fox, Sloan-Kettering Institute, New York; and acyclovir, kindly supplied by Dr. W. G. Wilson, Nippon Welcome Co., Shinoo, Osaka, Japan.

RESULTS

1. *Susceptibility of VZV*

As shown in Fig. 1, BV-araU markedly inhibited plaque formation of all ten strains of VZV; it caused complete inhibition at a concentration as low as 0.01 μg/ml, and had PD_{50} values of 0.0001 to 0.0007 μg/ml. Shigeta et al. (1983) also found that the PD_{50} values of BV-araU for 9 of 10 strains of VZV tested were about 0.001 μg/ml. Acyclovir was much less inhibitory, its average PD_{50} value for the VZV strains being 0.5 μg/ml. This value is similar to that reported by Crumpacker et al.

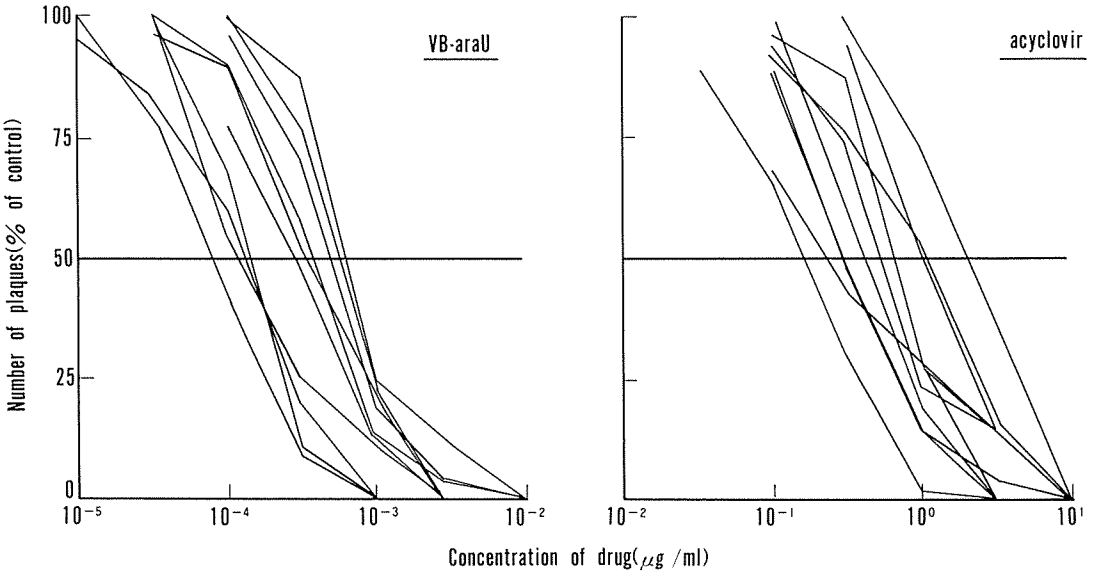

FIGURE 1. *Inhibitions by BV-araU and acyclovir of plaque formation by ten strains of VZV.*

(1979) but somewhat lower than that reported by Shigeta et al. (1983). The PD_{50} values, determined graphically, of all the compounds tested for the ten strains of VZV are listed in Table 1. All the strains of VZV were highly susceptible to not only BV-araU but also BV-araUMP and other XV-araUs, which had average PD_{50} values of less than 0.001 μg/ml. VZV strains were also susceptible to all the other thymidine analogues tested except ethyl-araU; surprisingly, most of the VZV strains were strongly resistant to ethyl-araU, to which HSV-1 was very susceptible (Machida et al., 1979).

The variation in susceptibilities of different strains of VZV to individual compounds was relatively large. Strains YS, Oka and Asahikawa were relatively less susceptible than the other strains to most of the compounds tested, whereas strains K 9, Ohtomo and Batson showed relatively high susceptibilities. The former strains were freshly isolated and corresponded to clinical isolates, whereas the latter strains were at high passage levels and might correspond to laboratory strains. However, strains YS, Oka and Asahikawa showed higher susceptiblities, and strains K 9, Ohtomo and Batson showed lower susceptibilities to IDU and aceto-dUrd. The ratio of the highest to the lowest PD_{50} values of these compounds for VZV strains ranged from 4 to 40, and were generally 5 to 20. These ratios were higher than those observed with HSV-1 and HSV-2 (Machida, 1986). While the strains varied in susceptibility, the relative order of antiviral potencies of the compounds against each strain of VZV was constant, except in the cases of IDU and aceto-dUrd mentioned above.

2. *Comparison with HSV*

For comparison of the susceptibilities of VZV and HSV to thymidine analogues, the PD_{50} values of each compound for three strains of HSV-1 and HSV-2, and three isolates typed as HSV-1 on HEL-F cells were determined. The method of determination and virus strains employed were described previously (Machida and Sakata, 1984; Machida, 1986). The PD_{50} values for each strain

TABLE 1. *Susceptibilities of VZV to thymidine analogs*

Compound	50%			
	YS	Asahikawa	Oka	Kawaguchi
IDU	0.84	0.65	0.27	0.32
EDU	10.0	18.2	22.9	3.8
BVDU	0.010	0.019	0.026	0.0058
aceto-dUrd	40	40	62	36
MCM-dUrd	16.2	10.2	4.9	5.8
OH-dUrd	2.3	4.0	19.7	38
araT	0.28	0.19	0.18	0.18
ethyl-araU	760	140	890	310
vinyl-araU	1.0	0.45	0.36	0.079
BV-araU	0.00063	0.00027	0 00037	0.00014
CV-araU	0.00093	0.00079	0 00054	0.00030
IV-araU	0.00071	0.00056	0.00028	0.00034
BV-araUMP	0.00040	0.00043	0.00094	0.00047
FIAC	0.045	0.0195	0.051	0.026
FMAU	0.0068	0.0214	0.010	0.0049
FVAU	0.027	0.030	0.017	0.034
acyclovir	1.12	1.78	1.02	0.60

[a] Geometric mean of 2-4 separate experimeriments.

TABLE 2. *Comparison of susceptibilities of HSV-1, HSV-2, HSV-1 isolates and VZV to thymidine analogues*

Compound	Average 50% plaque reduction dose (μg/ml)[a]			
	HSV-1	HSV-2	HSV-1 isolate	VZV
IDU	0.42	4.19	0.67	0.95
EDU	0.48	1.81	2.03	6.74
BVDU	0.014	9.8	0.024	0.008
aceto-dUrd	3.33	>1000	3.45	18.7
MCM-dUrd	8.0	88.5	14.7	2.66
OH-dUrd	22.7	16.2	13.4	15.0
araT	0.19	0.42	0.14	0.063
ethyl-araU	1.42	74	0.73	449
vinyl-araU	0.020	2.41	0.035	0.128
BV-araU	0.0094	13.1	0.012	0.00026
CV-araU	0.019	16.3	0.010	0.00043
IV-araU	0.010	6.9	0.010	0.00037
BV-araUMP	0.0089	18.3	0.015	0.00042
FIAC	0.012	0.017	0.023	0.015
FMAU	0.0045	0.010	0.016	0.0049
FVAU	0.014	0.423	0.015	0.029
acyclovir	0.034	0.055	0.046	0.50

[a] Geometric mean PD_{50} values for three strains of each HSV and ten strains of VZV.

Plaque reduction dose (μg/ml)[a]					
K 9	Hirai	Kanno	Ohtomo	Batson	CaQu
1.15	0.79	2.37	1.48	2.45	1.65
1.26	12.3	24.0	1.6	6.2	3.3
0.0058	0.0065	0.010	0.0024	0.0015	0.025
3.2	21.9	25.1	16.6	5.4	9.8
1.58	1.35	0.72	0.40	4.3	1.41
14.1	19.5	6.9	36	76	16.6
0.030	0.034	0.038	0.017	0.017	0.049
631	631	740	72	995	562
0.24	0.071	0.048	0.058	0.035	0.059
0.00015	0.00050	0.00034	0.00014	0.00008	0.00055
0.00021	0.00062	0.00145	0.00015	0.00013	0.00055
0.00023	0.00045	0.00056	0.00013	0.00036	0.00045
0.00024	0.00063	0.00063	0.00025	0.00023	0.00042
0.0048	0.0072	0.0132	0.0078	0.0158	0.0091
0.0025	0.0025	0.0066	0.0040	0.0032	0.0021
0.011	0.060	0.049	0.031	0.020	0.042
0.53	0.30	0.29	0.17	0.44	0.23

were presented previously (Machida, 6th International Congress of Virology, Sendai, 1985). In table 2, the susceptibilities of the strains to nucleoside analogues are compared in terms of PD_{50} values. VZV strains were much more susceptible than HSV-1 to XV-araUs and BV-araUMP, but they had the same susceptibility as HSV-1 or only slightly more to BVDU, MCM-dUrd, araT, F-araPyr and IDU. They were less susceptible than HSV-1 to EDU, ethyl-araU, vinyl-araU, aceto-dUrd and acyclovir; the average PD_{50} value of ethyl-araU for VZV was more than 300-fold that for HSV-1. HSV-2 is known to be significantly less susceptible than HSV-1 to certain nucleoside analogues such as BVDU, XV-araUs and ethyl-araU (De Clercq 1984; Machida et al., 1979; 1981). Furthermore, VZV was less susceptible than HSV-2 to ethyl-araU and EDU as well as acyclovir.

DISCUSSION

The development of a number of thymidine analogues that are active against VZV has been expected, because VZV as well as HSV is capable of inducing virus specific TK (Cheng et al., 1979; Ogino et al., 1977). Herpesvirus-induced TK can phosphorylate some thymidine analogues to their nucleoside monophosphate derivatives, whose triphosphate derivatives competitively inhibit viral DNA synthesis (De Clercq, 1984). Indeed, VZV have already been shown to be susceptible to some thymidine analogues, to which HSV is also susceptible. Although I found differences in the susceptibilities of different VZV strains tested, I also found some differences between the susceptibilities of VZV and HSV: i) VZV was much more susceptible than HSV-1 to BV-araU and related compounds, and ii) VZV was markedly less susceptible than HSV-1 to acyclovir, EDU, aceto-dUrd, ethyl-araU, vinyl-

araU and FVAU. Thus, VZV exhibited less susceptibility than HSV-1 to thymidine analogues having an ethyl or vinyl residue at the C-5 position of the pyrimidine base. On the other hand, interestingly, halogenation of the vinyl residual at the C-5 position resulted in increase in the antiviral potency against VZV, although significant antiviral activity against HSV-2 was lost by this chemical modification of vinyluracil (Machida et al., 1981).

ACKNOWLEDGEMENTS

The author wishes to thank Dr. A. Kuninaka for his support and for critical reading of the manuscript, and Miss M. Nishitani for excellent technical assistance. I also thank Dr. K. Yamanishi, Research Institute for Microbial Diseases, Osaka University, for recommending this paper for publication in this journal.

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