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Structure-activity relationship of the affinity of 5-substituted uracil nucleoside analogues for varicella-zoster virus thymidine kinase and their activity against varicella-zoster virus

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Abstract

We investigated structure-activity relationships of 5-substituted uracil nucleoside analogues for their selective antiviral activity against varicella-zoster virus (VZV) and affinity for VZV thymidine kinase (TK). Anti-proliferative activity of the compounds was measured using human lymphoblastoid cells. Most 2′-deoxyribofuranosyluracil, arabinofuranosyluracil (araU) and 2′-deoxy-2′-fluoro-arabinofuranosyluracil derivatives showed selective anti-VZV activity as well as activity against herpes simplex virus types 1 and 2. 2′-Deoxyuridine derivatives showed higher affinity than the corresponding araU analogues. A correlation was seen between the 50% effective doses for VZV and the K_i values for VZV TK, except for 5-ethyl-2′-deoxyuridine and 5-ethyl araU that showed relatively high affinity for VZV TK without showing any activity against VZV. 5-Halogenovinyluracil nucleosides showed the highest affinity and the most potent and selective anti-VZV activity. 2′-Deoxy-2′-fluoro-arabinofuranosyluracil derivatives exhibited high anti-VZV potency though they showed relatively low affinity for VZV TK. Some 3′-deoxythymidine analogues having anti-human immunodeficiency virus activity were inactive against herpesviruses. © 1997 Elsevier Science B.V.

Keywords: Varicella-zoster virus; Varicella-zoster virus thymidine kinase; 5-substituted uracil nucleosides; BV-araU

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1. Introduction

Varicella-zoster virus (VZV) encodes a thymidine kinase (TK) having a biochemical behavior similar to that induced by the herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) (Ogino et al., 1977; Cheng et al., 1979) and VZV TK has amino acid sequence homology in the substrate binding regions with HSV TKs (Balasubramanian et al., 1990). These herpesvirus-induced kinases have broad substrate specificities and phosphorylate a number of selective antiviral nucleoside analogues which are scarcely or cannot be phosphorylated by cellular kinase(s). Indeed, the sufficiency of nucleoside analogues as substrates for viral TK correlates with their anti-herpesvirus activity and the critical role of viral TKs in their activation is the rationale for the development of selective anti-herpesvirus nucleoside analogues as demonstrated by the phosphorylation and anti-HSV-1 activity of acyclic purine nucleoside analogues and 5-(2-halogenovinyl)-2′-deoxyuridines (Keller et al., 1981; Cheng et al., 1981). On the other hand, marked differences have been shown in the antiviral spectra of nucleoside analogues: 1-β-D-arabinofuranosyl-5-(*E*-2-bromovinyl)uracil (BV-araU) and 5-(*E*-2-bromovinyl)-2′-deoxyuridine (BVDU) exhibt particularly potent antiviral activity against VZV but have little activity against HSV-2, while acyclovir is much less active against VZV than against HSV-1 and HSV-2 (Machida, 1986, 1990; Shigeta et al., 1983). The 5-prop-1-ynyl derivative of 1-β-D-arabinofuranosyluracil (araU) also exhibits significant anti-VZV activity without activity against HSV-1 (Rahim et al., 1992). There are some differences in the substrate specificities of the viral TKs despite of the similarity in structural and biochemical features and the differences in the antiviral spectra may reflect, at least in part, differences in the substrate specificities of the viral kinases. The thymidine analogues that are particularly selective against VZV have been considered as candidates of novel anti-VZV agents. In clinical trials, oral BV-araU effectively reduced the time of vesicle formation, erythema and pain in immunocompromised patients with herpes zoster (Hiraoka et al., 1991) and BVDU was found to be effective in the treatment of VZV infection in immuno-compromised children (Heidl et al., 1991).

To study structure-activity relationships, we tested 5-substituted uracil nucleoside analogues for their selective antiviral activity against VZV in comparison with anti-HSV-1 and HSV-2 activities and their anti-proliferative activity using human lymphoblastoid leukemia cells. We also measured the inhibition constants of the nucleoside analogues for VZV TK using bacterially expressed enzyme.

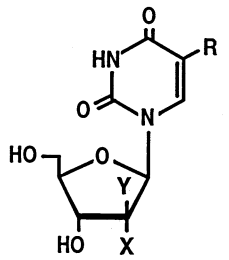
2. Materials and methods

2.1. Cells and viruses

A human embryonic lung (HEL) cell line, HAIN-55, a gift from Dr Okumura, (National Institute of Health of Japan, Tokyo) and the VZV Oka strain, the HSV-1 VR-3 strain and the HSV-2 MS strain were used for the antiviral activity tests. The origin of viruses has been described previously (Machida, 1990). Human T-cell acute lymphoblastoid leukemia cells, CCRF-HSB-2, were used for the anti-cell growth activity test.

2.2. Compounds

The following pyrimidine nucleoside analogues were synthesized at the Chemistry Laboratory of Yamasa Corporation or were commercial products of the company: Thymidine (Thd), BVDU, 5-ethyl, 5-vinyl, 5-ethynyl, 5-prop-1-ynyl and 5-iodo derivatives of 1-β-D-2′-deoxyribofuranosyluracil (abbreviated to Et-dU, Vinyl-dU, Ethy-dU, Prpy-dU and IDU, respectively), 1-β-D-arabinofuranosylthymine (araT), BV-araU, 5-ethyl, 5-vinyl, 5-(*E*-2-chlorovinyl), 5-(*E*-2-iodovinyl), 5-(2,2-dibromovinyl), 5-ethynyl, 5-prop-1-ynyl and 5-iodo derivatives of araU (abbreviated to Et-araU, Vinyl-araU, CV-araU, IV-araU, DiBrV-araU, Ethy-araU, Prpy-araU and I-araU, respectively), 5-methyl, 5-vinyl, and 5-(*E*-2-bromovinyl) derivatives of 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (abbreviated to FMAU, FVAU and FBVAU, respectively), 2′,3′-dideoxythymidine (DDT), 3′-azido-3′-deoxythymidine



X = Y = H : 5-Substituted 2'-deoxyribofuranosyluracils (2'-deoxyuridine series)
X = H, Y = OH : 5-Substituted arabinofuranosyluracils (araU series)
X = H, Y = F : 5-Substituted 2'-deoxy-2'-fluoroarabinofuranosyluracils (F-araU series)
X = OH, Y = H : 5-Substituted ribofuranosyluracils

Fig. 1. Structures of 5-substituted uracil nucleoside analogues.

(AZT), 2′,3′-didehydro-2′,3′-dideoxythymidine (D4T), 3′-deoxy-araT (3d-araT) and 5-(*E*-2-bromovinyl)uridine (BV-riboU). Synthesized analogues were identified by NMR and their mass spectra and their purity was determined to be 98% or more by HPLC. 1-(2-Deoxy-2-fluoro-β-D-arabinofuranosyl)-5-ethyluracil (FEAU) and 5-iodo-1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)uracil (FIAU) were gifts from Dr J.J. Fox, Sloan-Kettering Institute, New York. The structures of these compounds are given in Fig. 1.

2.3. Antiviral activity tests

Antiviral activity against VZV, HSV-1 and HSV-2 was determined by the plaque reduction method as described previously (Machida and Nishitani, 1990; Machida et al., 1991). Briefly, confluent monolayers of HEL cells grown in a 12 multi-well plate (Corning Glass Works, Corning, New York) were infected with 50–100 plaque forming units of cell-associated VZV Oka strain, HSV-1 VR-3 strain or HSV-2 MS strain. Drugs were added to the VZV-infected cultures after virus adsorption for 1 h at 37°C in duplicate for each dilution of drug, or added to HSV-1- or HSV-2-infected cells, after virus adsorption for 30 min at 37°C. After the virus suspension had been discarded, the infected cells were overlaid with maintenance medium containing 0.8% methyl-cellulose (Nacalai Tesque, Tokyo). The VZV-infected cells and HSV-1- or HSV-2-infected cells were incubated in a 5% CO_2-air incubator at 37°C for 4 days and for 2–3 days, respectively and then stained with a 0.5% crystal violet solution. The number of VZV and HSV plaques were counted under a stereoscopic light microscope. Dilutions of drugs were made in serial half-$\log_{10}$ decrement with maximum concentrations of 160 and 320 μg/ml, for VZV-infected and HSV-infected cultures, respectively. A total of four to five drug concentrations (1.5–2 $\log_{10}$ range) were used for each drug and virus combination in the plaque reduction assay. Percent inhibition of plaque formation, compared with plaque number in drug-untreated control cultures, were calculated for each drug concentration used. The drug dosage required to reduce plaque formation by 50% (ED_{50}) was determined by interpolation from the dose-response curve which was obtained graphically for each test drug.

2.4. Anti-proliferative activity test

Inhibitory effect of test compounds on cell growth was determined by the MTT assay using human T-cell acute lymphoblastoid leukemia cells, CCRF-HSB-2, as described previously (Miura et al., 1996) with some modifications. A 90 μl volume of RPMI 1640 medium supplemented with 10% fetal bovine serum containing 5×10^3 of CCRF-HSB-2 cells was seeded into each well of a 96-well flat bottom microplate (NUNC, Roskilde,

Denmark). A 10 μl volume of drug solution, prepared in serial four-fold dilution with maximum concentrations of 1.6 mg/ml, was added simultaneously in triplicate to each well. The plate was incubated at 37°C in a humidified atmosphere of 5% CO_2. After 72 h of incubation, 10 μl of MTT solution (5 mg/ml in phosphate-buffered saline lacking calcium and magnesium) was added to each well and cells were incubated for additional 4 h at 37°C, the 100 μl of 50% dimethylformamide (VV) and 20% SDS (WV) dissolved in 0.02 N HCI was added to solubilize any MTT-formazan formed. The optical density at 570 nm (OD_{570}) of each well was measured with an Immuno-Reader NJ-2000 (InterMed Japan, Tokyo, Japan) and the inhibition of cell growth (%) was calculated using the following formula: Inhibition of cell growth (%) = $(1 - T/C) \times 100$, where C is the mean OD_{570} of the control group and T is the mean OD_{570} of the treated group. The 50% inhibitory concentration (IC_{50}) was determined from the dose-response curves.

2.5. Methods for preparation of VZV TK and determination of the inhibition constant for VZV TK

VZV TK was cloned into an expressing vector pKK 223-3 (pKK-VZTK) and was expressed in the TK deficient *E. coli* strain C600 TK^- as described previously (Suzutani et al., 1992). The bacterially expressed VZV TK had the same biological characteristics as those of native VZV TK (Suzutani et al., 1993). The bacterially expressed VZV TK was induced using isopropyl-β-D-thiogalactopyranoside and a crude VZV TK cell extract was prepared and VZV TK was partially purified by ammonium sulfate precipitation as reported previously (Cheng et al., 1979). The partially purified enzyme solution was loaded onto a DE52 (Pharmacia, Uppsala, Sweden) column and eluted with a linear gradient of 0–2 M KCl. The peak fractions of TK activity were collected and dialyzed against 10 mM Tris–HCl buffer pH 7.5 containing 5 mM 2-mercaptoethanol and 10% glycerol. The resultant solution contained TK with specific activity of 1184 units/mg protein, which was about 2000-fold purified from cell extract. The enzyme solution was used for the TK assay according to the method of Lee and Cheng (1976) with some modifications. The reaction mixture contained 25 mM Tris–HCI pH 7.5, 2 mM ATP, 2 mM $MgCl_2$, 3 mM creatine phosphate, 0.5 mg of bovine serum albumin, 25 mM NaF, 1 unit of creatine kinase and 150 nmol of [methyl-^{3}H]Thd (6.7 Ci/mmol; NEN Research Products, Wilmington, DE), in a final volume of 0.1 ml and was incubated at 37°C for 30 min. In these conditions the reaction progressed linearly for at least 60 min. The product of the reaction [methyl-^{3}H]TMP was separated by adsorption onto DE81 paper disk (Whatman, Kent, UK) and radioactivity was measured with a scintillation counter (Aloka LSL-35000, Tokyo). The TK inhibition assay was performed for compounds which showed anti-herpesvirus activities under the same conditions for the TK assay except that the test compound was added to the reaction mixture in duplicate at a concentration of 0.1–20 μM. Inhibition constants (apparent K_i) of the test compounds for VZV TK were calculated by the double reciprocal plot method (Lineweaver–Burk's plot) from data using three to five, usually four, concentrations of inhibitors as described previously (Yokota et al., 1989).

3. Results

3.1. Anti-herpesvirus activities

As shown in Table 1, 5-substituted 2′-deoxyuridine, araU and 2′-deoxy-2′-fluoroarabinofuranosyluracil (F-araU)-analogues all showed potent to modest anti-VZV effects, except for Et-dU and Et-araU. These compounds, other than Prpy-araU and DiBrV-araU, also showed antiviral activities against HSV. 5-(2-Halogenovinyl) and 5-alkynyl uracil nucleosides exhibited more potent activity against VZV than against HSV-1 (ED_{50} for HSV-1 divided by ED_{50} for VZV was almost ten or greater) and 5-methyluracil analogues exhibited anti-VZV activity almost equivalent to anti HSV-1 activity. In contrast, Et-dU and Et-araU were inactive against VZV, while they showed marked anti-HSV-1 activity.

Table 1
Antiviral and anti-cell growth activities of 5-substituted uracil nucleosides with various sugars

Compound		50% Plaque reduction dose (μM)[a]			Ratio of anti-HSV-1 to anti-VZV[c]	ID_{50} for cell growth (μM)[d]	Selectivity index for VZV[e]
Sugar	Base (R =)[b] (Abbreviation)	HSV-1	HSV-2	VZV			
2-Deoxyribose	$-CH_2CH_3$ (Et-dU)	32	39	>625	<0.05	8.2	<0.01
	$-CH{=}CH_2$ (Vinyl-dU)	0.43	2.80	1.18	0.36	17	15
	$-CH{=}CHBr$ (BVDU)	0.117	577	0.043	2.7	135	3100
	$-C{\equiv}CH$ (Ethy-dU)	2.54	9.7	0.40	6.4	8.7	22
	$-C{\equiv}CHCH_3$ (Prpy-dU)	76	380	3.2	24	312	98
	$-I$ (IDU)	2.63	4.12	0.99	2.7	76	77
Arabinose	$-CH_3$ (araT)	1.78	1.74	1.43	1.2	58	40
	$-CH_2CH_3$ (Et-araU)	7.4	>243	>588	<0.01	>588	—
	$-CH{=}CH_2$ (Vinyl-araU)	0.37	16.7	4.07	0.09	>592	>140
	$-CH{=}CHBr$ (BV-araU)	0.060	138	0.0023	26	>406	>200 000
	$-CH{=}CHBr_2$ (DiBrV-araU)	704	>751	18.8	37	>376	>20
	$-CH{=}CHCl$ (CV-araU)	0.086	513	0.0056	15	>526	>94 000
	$-CH{=}CHI$ (IV-araU)	0.119	96	0.0035	34	>404	>110 000
	$-C{\equiv}CH$ (Ethy-araU)	7.5	57	1.01	7.4	23	23
	$-C{\equiv}CHCH_3$ (Prpy-araU)	>1135	>1135	1.63	>700	>567	>350
	$-I$ (I-araU)	23.5	31	70	0.34	>423	>6
2-Fluoroarabinose	$-CH_3$ (FMAU)	0.081	0.162	0.108	0.75	1.5	14
	$-CH_2CH_3$ (FEAU)	0.22	0.77	0.48	0.46	>584	>1200
	$-CH{=}CH_2$ (FVAU)	0.074	1.10	0.31	0.24	114	370
	$-CH{=}CHBr$ (FBVAU)	0.140	24	0.023	6.0	413	18 000
	$-I$ (FIAU)	0.073	0.161	0.212	0.34	3.1	15
Ribose	$-CH{=}CHBr$ (BV-riboU)	>917	>917	42	>22	292	7
3-Deoxyarabinose	$-CH_3$ (3d-araT)	>1322	>1322	32	>41	>661	>20

[a] Tests for anti-VZV activity of all compounds and for anti-HSV activities of some key compounds were conducted two to three times. Other tests were single experiments.
[b] Structures of compounds are given in Fig. 1.
[c] ED_{50} for HSV-1 divided by ED_{50} for VZV.
[d] Data from a single experiment for many test compounds except for some key compounds for which two experiments were conducted.
[e] ID_{50} for cell growth divided by ED_{50} for VZV.

5-Vinyl and 5-iodouracil nucleoside analogues, except for IDU, were less potent against VZV than against HSV-1. The araU analogues were generally as active or less active against HSV-1 and HSV-2 than the corresponding 2′-deoxyuridine analogues, while some of the former showed more potent anti-VZV activity than the corresponding 2′-deoxyuridine analogues. This depended on the substitution of the 5-position of uracil. F-araU analogues exhibited marked activities against all three herpesviruses tested irrespective of the substituent at the 5-position. Thymine nucleoside analogues other than araT and FMAU, such as AZT, DDT and D4T, which were reported to have potent anti-human immunodeficiency virus activity, were inactive against the three herpesviruses (data not shown). 3d-araT and BV-riboU showed no activity against HSV, but showed modest anti-VZV activity.

3.2. Antiproliferative activity and selectivity of drugs

5-Substituted araU analogues did not show any detectable anti-cell growth activity against human T-cell acute lymphoblastoid leukemia cells, except for araT and Ethy-araU that had weak activity (Table 1). In contrast, the antiproliferative activity of the 2′-deoxyuridine derivatives varied with the substituent at the C-5 position. Et-dU and Ethy-dU were active, while BVDU and Pryp-dU were very weakly active. Anti-proliferative activity of F-araU analogues also strongly depended on the substituent at the C-5 position; FMAU and FIAU strongly inhibited cell growth, while other 2′-fluoro nucleosides showed very weak or no activity. Highly selective anti-VZV activity was noted for the 5-halogenovinyluracil nucleosides.

3.3. Affinity for VZV TK and correlation with anti-VZV activity

Apparent K_i values for VZV TK determined in the phosphorylation of Thd of compounds exhibiting anti-herpesvirus activities ranged from 0.12–6.30 μM (Table 2). As these compounds showed competitive inhibition of the enzyme and the K_i values reflected the binding affinity for VZV TK, indicating that these analogues had high or moderate affinity for VZV TK. The degree of affinity for the enzyme was strongly affected by both sugar moieties and substitutions at the 5-position of uracil. 2′-Deoxyuridine analogues showed higher affinity than the corresponding araU analogues except for araT, which showed a K_i value lower than the apparent K_m value of VZV TK for Thd. Fluorination at the 2′-position of the sugar moiety resulted in marked

Table 2
Inhibition constants for VZV TK of 5-substituted uracil nucleosides

Compound		K_i for VZV TK[a] (μM)
Sugar	Base (R =)[b] (Abbreviation)	
2-Deoxyribose	$-CH_3$ (Thd)	0.79[c]
	$-CH_2CH_3$ (Et-dU)	0 47
	$-CH{=}CH_2$ (Vinyl-dU)	0.41
	$-CH{=}CHBr$ (BVDU)	0.12
	$-C{\equiv}CH$ (Ethy-dU)	1.56
	$-C{\equiv}CHCH_3$ (Prpy-dU)	2.30
	–I (IDU)	1.06
Arabinose	$-CH_3$ (araT)	0.46
	$-CH_2CH_3$ (Et-araU)	4.99
	$-CH{=}CH_2$ (Vinyl-araU)	6.30
	$-CH{=}CHBr$ (BV-araU)	0.25
	$-CH{=}CHBr_2$ (DiBrV-araU)	4.05
	$-CH{=}CHCl$ (CV-araU)	0.42
	$-CH{=}CHI$ (IV-araU)	0.28
	$-C{\equiv}CH$ (Ethy-araU)	4.90
	$-C{\equiv}CHCH_3$ (Prpy-araU)	5.19
2-Fluoroarabinose	$-CH_3$ (FMAU)	3.06
	$-CH_2CH_3$ (FEAU)	1.14
	$-CH{=}CHBr$ (FBVAU)	2.75
Ribose	$-CH{=}CHBr$ (BV-riboU)	4.48

[a] K_i values were determined from two TK inhibition assays.
[b] Structures of compounds are given in Fig. 1.
[c] Michaelis-Menten constant (K_m)

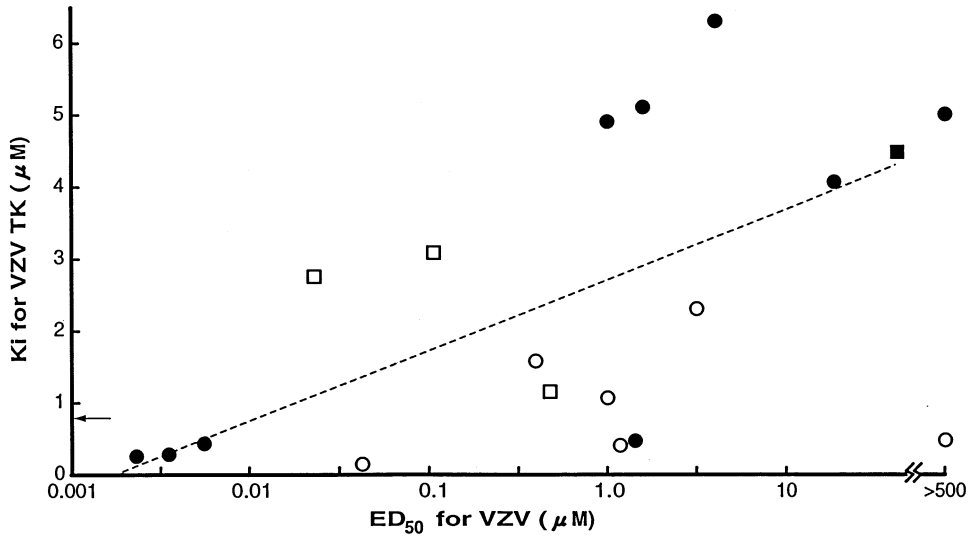


Fig. 2. Correlation between anti-VZV effects and the inhibition constant for VZV TK of 5-substituted uracil nucleosides. ○, 2′-deoxyuridine series; ●, araU series; □, F-araU series; and ■, BV-riboU. The dotted line indicates the linear regression calculated from $\log_{10}$ ED_{50} and K_i values excluding those for Et-dU and Et-araU. The arrow shows K_m value of VZV TK for Thd.

lower affinity, except for FEAU which showed higher affinity than Et-araU. Substitution with a bromovinyl or an iodovinyl group at the 5-position was particularly sufficient for high affinity for the enzyme, and BVDU showed the highest affinity. The order in the affinity for the 5-position substitution in the araU series was: bromovinyl-, iodovinyl- > chlorovinyl-, methyl- ≫ dibromovinyl- > ethyl-, ethynyl- > propynyl- > vinyl-. A similar activity-relationship was seen for the 2′-deoxyuridine series, although ethyl and vinyl derivatives showed higher affinity than Thd. DiBr-araU and BV-riboU were only marginally effective against VZV and they also showed lower affinity for the VZV TK.

Compounds showing high affinity for VZV TK generally exhibited potent anti-VZV activity. Exceptions were Et-dU and Et-araU which showed relatively high affinity for the enzyme with K_i values of 0.47 and 4.99 μM, respectively, but had no anti-VZV activity. A clear correlation was seen between the anti-VZV potency expressed as $\log_{10}$ ED_{50} and the K_i values for all compounds (Fig. 2; the correlation coefficient = 0.608 was calculated from these values except for Et-dU and Et-araU) and for araU and the 2′-deoxyuridine series. The correlation coefficients were 0.767 and 0.684 for araU and the 2′-deoxyuridine series, respectively. On the other hand, F-araU analogues except for FBVAU showed greater anti-VZV activity than the corresponding 2′-deoxyuridine and araU analogues, but they showed much lower affinity for VZV TK and a correlation was not established when compared with nucleoside analogues having the same substitution at the 5-position with different sugar moieties.

4. Discussion

A number of 5-substituted uracil nucleoside analogues act as thymidine analogues and show selective anti-herpesvirus effects. Phosphorylation by herpesvirus-induced TKs is essential for their intracellular activation and the exhibition of antiviral actions. The present study reveals that many 5-substituted uracil nucleosides have no or weak inhibitory effects on cell growth. These results, taken together with the results of the anti-VZV activity tests, confirm that the anti-VZV activity of the uracil nucleosides is selective. We have previously observed that antiherpesviral 5-

substituted araU analogues such as 5-(2-halogenovinyl) and 5-propynyl arabinofuranosyluracils did not show any antiproliferative activity against the HEL cells, while some 2′-deoxyuridine and F-araU analogues such as IDU, FMAU and FVAU showed a marked to moderate inhibitory action on the growth of the cells (Machida, 1986; Machida et al., 1995). The trend in structure-antiproliferative activity relationship of the 5-substituted uracil nucleosides was similar to those observed here using a different cell line. In addition, previous work has revealed considerable variation in the antiviral spectra of nucleoside analogues (Machida, 1986; Rahim et al., 1992), while similarity exists in structural and biochemical features of the HSV and VZV TKs. Our data on the structure-activity relationship of the antiviral activities revealed differences in the antiviral spectra of the uracil nucleoside analogues and demonstrated superiority of some 5-substituted araU analogues over the corresponding 2′-deoxyuridine analogues in anti-VZV potency and selectivity.

All anti-herpesvirus uracil nucleoside analogues tested showed high or moderate affinity for VZV TK and some showed K_i values lower than the apparent K_m of VZV TK for Thd. The 2′-deoxyuridine analogues showed higher affinity than the corresponding araU analogues, agreeing with the findings of Roberts et al. (1993) that 2′-deoxyribofuranosyl nucleoside analogues were more sufficient as substrates for VZV TK than the corresponding araU analogues. Affinity for VZV TK was lowered by fluorination at the 2′-position, except for the 5-ethyluracil nucleosides. The degree of affinity was also strongly affected by substitutions at the 5-position of uracil. In both the 2′-deoxyuridine and araU series, sufficient affinity for VZV TK of compounds having halogenovinyl group substitution at the 5-position was noteworthy and decreased affinity by substitution with alkynyl groups was observed. Compounds showing higher affinity for VZV TK tended to exhibit more potent anti-VZV activity and a correlation was seen between anti-VZV activity and affinity for VZV TK, except for Et-dU and Et-araU and the correlation observed in the araU series was clearer than that observed in the 2′-deoxyuridine series and that for all compounds. On the other hand, F-araU analogues showed relatively low affinity and there was no correlation when compared with nucleoside analogues having the same substitution at the 5-position with different sugar moieties.

The highest affinity for VZV TK and the most potent anti-VZV activity were noted for BVDU, BV-araU and IV-araU. 5-Bromovinyluracil nucleoside analogues other than those described here also show highly potent and selective anti-VZV activity (Dyson et al., 1991; Slusarchyk et al., 1992; Machida et al., 1993). The bromovinyl substitution at the 5-position of uracil nucleoside analogues may be highly adequate for affinity for VZV TK. BVDU showed higher affinity than BV-araU. We confirmed this finding using native VZV TK (Yokota et al., 1989), while Roberts et al. (1993) reported that the K_i value of BV-araU for VZV TK was lower than that of BVDU. Inhibition of viral DNA polymerase by antiviral nucleoside triphosphate is another critical role for antiviral action of nucleosides. BV-araU triphosphate inhibits VZV DNA polymerase as strongly as BVDU triphosphate (Yokota et al., 1989). Factors other than the affinities for VZV TK and the inhibitory effect of the triphosphates on VZV DNA polymerase may also contribute to the anti-VZV potency of uracil nucleoside analogues. One of these factors may be the phosphorylation of the nucleoside monophosphates by viral TK which may have an important role in the activation of some selective nucleoside analogues (Fyfe, 1982).

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