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Differential Affinities of 5-(2-Halogenovinyl)-2′-Deoxyuridines for Deoxythymidine Kinases of Various Origins

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SUMMARY

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The inhibition constants (K_i) of a series of 5-(2-halogenovinyl)-2′-deoxyuridines, including *E*-5-(2-chlorovinyl)-dUrd, *E*-5-(2-bromovinyl)-dUrd, *E*-5-(2-iodovinyl)-dUrd, and *Z*-5-(2-bromovinyl)-dUrd, have been determined for the dThd kinases of human cytosol or mitochondria, herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2), or varicella-zoster virus (VZV). The *E*-5-(2-halogenovinyl)-2′-deoxyuridines had a much higher affinity for HSV-1 (or VZV) dThd kinase than for HSV-2 dThd kinase, and the affinity of *E*-5-(2-bromovinyl)-dUrd for HSV-1 dThd kinase was also much higher than that of its stereoisomer, *Z*-5-(2-bromovinyl)-dUrd. The relative affinities of these compounds for the virus-induced dThd kinase correlated closely with their inhibitory effects on these viruses, suggesting that the selectivity of *E*-5-(2-bromovinyl)-dUrd and its congeners toward herpes viruses (HSV and VZV) is to a large extent dependent on their phosphorylation by the virus-induced dThd kinase.

The *E*-5-(2-halogenovinyl)-2′-deoxyuridines, *E*-5-(2-chlorovinyl)-dUrd, *E*-5-(2-bromovinyl)-dUrd, and *E*-5-(2-iodovinyl)-dUrd belong to the most potent and most selective antiherpes compounds that have been discovered to date. These compounds inhibit the replication of HSV-1[5] in primary rabbit kidney cell cultures and the replication of VZV in human diploid cell cultures at a concentration of about 0.01 μg/ml, while not affecting normal cell metabolism at concentrations up to 50–100 μg/ml (1–4). The most active congener of this series of compounds, *E*-5-(2-bromovinyl)-dUrd, has been found effective in the local and systemic treatment of HSV-1 skin lesions, and in reducing the associated mortality, in athymic nude mice (1, 5, 6), and in the local treatment of HSV-1 keratitis in rabbits (7, 8); preliminary clinical trials indicate that *E*-5-(2-bromovinyl)-dUrd may also be efficacious in the local treatment of HSV-1 keratitis (9) and the oral treatment of herpes zoster (4) in humans.

The *E* (entgegen) configuration appears to be essential for the antiherpes activity of *E*-5-(2-halogenovinyl)-2′-deoxyuridines, since the *Z* (zusammen)-isomer of *E*-5-(2-bromovinyl)-dUrd is significantly less active against HSV-1 than is the *E*-isomer (10). The *E*-5-(2-halogenovinyl)-2′-deoxyuridines are about 100–200 times less inhibitory to HSV-2 than to HSV-1 (2, 3) and are virtually inactive against a dThd kinase-deficient mutant of HSV-1. The latter finding (2, 3) suggests that the compounds must be phosphorylated by the virus-induced dThd kinase to exert their antiherpes activity. To obtain direct evidence for the role of the virus-induced dThd kinase in the anti-HSV-1 activity of *E*-5-(2-halogenovinyl)-2′-deoxyuridines, and in an attempt to unravel the molecular bases for the differential activity of *E*- and *Z*-5-(2-bromovinyl)-dUrd against HSV-1 and for the differential susceptibility of HSV-1 and HSV-2 toward *E*-5-(2-halogenovinyl)-2′-deoxyuridines, we have now determined the substrate affinity of these compounds for several dThd kinases, including those encoded by HSV-1 and HSV-2. Our data indicate that the differences in the anti-

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[5] The abbreviations used are: HSV-1 and HSV-2, herpes simplex virus types 1 and 2, respectively; VZV, varicella-zoster virus.

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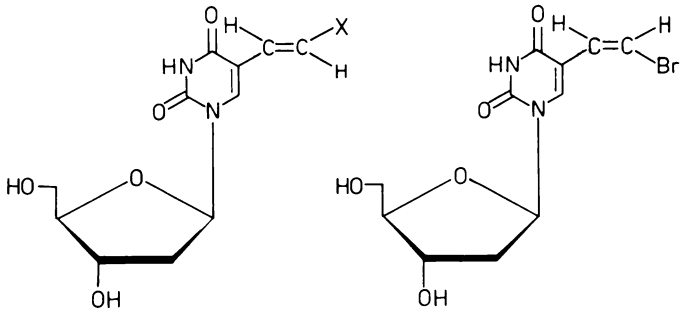

X = Cl : E-5-(2-chlorovinyl)-dUrd Z-5-(2-bromovinyl)-dUrd
Br : E-5-(2-bromovinyl)-dUrd
I : E-5-(2-iodovinyl)-dUrd

FIG. 1. *Structural formulae of 5-(2-halogenovinyl)-2'-deoxyuridine*

HSV-1 activity of *E* and *Z*-5-(2-bromovinyl)-dUrd, as well as the differences in the susceptibility of HSV-1 and HSV-2 toward the *E*-5-(2-halogenovinyl)-dUrds, may reside in the differential affinity of these compounds for the corresponding virus-induced dThd kinases. From these data, one may also infer that the HSV-1 dThd kinase (and the VZV dThd kinase) possesses a unique binding site that specifically recognizes the *E*-configuration of 5-(2-halogenovinyl)-2'-deoxyuridines.

The structural formulae of the test compounds are presented in Fig. 1. Their synthesis and physical characteristics have been described previously (10, 11). Their antiherpes activity has also been the subject of previous studies (2, 3, 10) and is reviewed in Table 1.

The human cytosol and mitochondrial dThd kinases were isolated from the peripheral blast cells of a leukemic patient. HSV-1 and HSV-2 dThd kinases were extracted from dThd kinase-deficient HeLa (BU 25) cells infected with either HSV-1 (strain KOS) or HSV-2 (strain 333). VZV dThd kinase was prepared from VZV-infected human foreskin cell cultures. The detailed procedures for the extraction and purification of the dThd kinases have been described previously (12–14).

To estimate the inhibition constants of the test compounds for the dThd kinase (Table 2), two concentrations of [^{14}C]dThd, 0.3 and 0.6 mM, were employed. The concentration of ATP-Mg^{2+} was 2 mM. Otherwise, the conditions for the enzyme assay were identical with those described by Lee and Cheng (12). To determine the

TABLE 1

Inhibitory effects of 5-(2-halogenovinyl)-2'-deoxyuridines on HSV-1 and HSV-2 primary rabbit kidney cell cultures

Compound	Minimal inhibitory concentration[a]		
	HSV-1	HSV-2	Reference
	μg/ml		
E-5-(2-Chlorovinyl)-dUrd	0.02	2	(3)
E-5-(2-Bromovinyl)-dUrd	0.008	1	(2, 3)
E-5-(2-Iodovinyl)-dUrd	0.012	2	(2, 3)
Z-5-(2-Bromovinyl)-dUrd	1	2	(10)

[a] ID_{50}: Dose necessary to inhibit 50% of the plaque-forming units.

ability of the test compounds to act as substrates for the dThd kinases (Table 3), we applied the procedure of Dobersen and Greer (16). The concentration of [γ-^{32}P]-ATP was 0.5 mM and the concentration of the 5-(2-halogenovinyl)-2'-deoxyuridines was 0.4 mM; [γ-^{32}P]ATP was obtained from New England Nuclear Corporation (Boston, Mass.).

The K_i values of the 5-(2-halogenovinyl)-2'-deoxyuridines for the various dThd kinases are presented in Table 2. The apparent K_i values obtained for the viral enzymes reflect the Michaelis-Menten constants (K_m) of the compounds, since they are good substrates for these enzymes (Table 3). It appears that all *E*-isomers had a lower K_i, and hence higher affinity, for the HSV-1 than for the HSV-2 dThd kinase. In addition, *E*-5-(2-bromovinyl)-dUrd had a remarkably high affinity for the VZV dThd kinase. The affinity of *Z*-5-(2-bromovinyl)-dUrd for HSV-1 dThd kinase was 10-fold lower than that of the *E*-isomer. None of the nucleoside analogues tested appeared to bind to the cytosol dThd kinase. The *E*-analogues had a rather high, and the *Z*-analogues a rather low, affinity for the mitochondrial dThd kinase.

Although the inhibition constant of *E*-5-(2-bromovinyl)-dUrd for the mitochondrial dThd kinase was rather low (0.83 μM), it was phosphorylated at a rate that was only one-third of the rate of dThd phosphorylation (Table 3). The rates of phosphorylation of the 5-(2-halogenovinyl)-dUrds by the HSV-1 and HSV-2 dThd kinases were similar to or even higher than that of dThd. Since the rates of phosphorylation were examined at a saturating concentration (0.4 mM) of the nucleoside analogue, they actually reflect V_{max}. Although all halogenovinyl derivatives were effectively phosphorylated by

TABLE 2

Inhibition constants of 5-(2-halogenovinyl)-2'-deoxyuridines for various kinds of dThd kinase

The dThd kinases were purified as described previously (12–14) and the K_i values (mean ± standard deviation) were determined by the procedure of Cheng and Prusoff (15). The amount of enzyme used was 0.02, 0.05, 0.1, 0.1, or 0.02 units for human cytosol, human mitochondrial, HSV-1 (KOS), HSV-2 (333), and VZV dThd kinase, respectively. Volume of reaction mixture was 0.1 ml.

Compound	Enzyme source				
	Human		HSV-1 (strain KOS)	HSV-2 (strain 333)	VZV
	Cytosol	Mitochondria			
			K_i (μM)		
E-5-(2-Chlorovinyl)-dUrd	>150	0.29 ± 0.13	0.24 ± 0.25	2.00 ± 0.30	—
E-5-(2-Bromovinyl)-dUrd	>150	0.83 ± 0.12	0.24 ± 0.09	4.24 ± 1.30	0.07 ± 0.01
E-5-(2-Iodovinyl)-dUrd	>150	1.08 ± 0.22	0.27 ± 0.04	5.71 ± 1.49	—
Z-5-(2-Bromovinyl)-dUrd	>150	34.8 ± 11.5	2.47 ± 0.98	2.54 ± 0.7	—

TABLE 3

Relative phosphorylation rates of 5-(2-halogenovinyl)-2′-deoxyuridines by various kinds of dThd kinase

The enzymes were purified as described previously (12–14). The amount of enzyme used was the same as indicated in footnote to Table 2. Values are means ± range.

Compound (0.4 mM)	Enzyme source			
	Human		HSV-1 (strain KOS)	HSV-2 (strain 333)
	Cytosol	Mitochondria		
	velocity (%) relative to dThd			
dThd	100	100	100	100
E-5-(2-Chlorovinyl)-dUrd	<5	48 ± 1	96 ± 8	112 ± 7
E-5-(2-Bromovinyl)-dUrd	<5	33 ± 3	90 ± 9	127 ± 11
E-5-(2-Iodovinyl)-dUrd	<5	24 ± 8	111 ± 22	155 ± 21
Z-5-(2-Bromovinyl)-dUrd	<5	15 ± 3	112 ± 30	184 ± 19

the viral enzymes, none served as an alternative substrate for the cytosol dThd kinase.

From the results presented in Tables 2 and 3, one may deduce that the cytosol dThd kinase does not bind and does not phosphorylate the 5-(2-halogenovinyl)-2′-deoxyuridines. However, these compounds act as alternative substrates for the HSV-1 and HSV-2 dThd kinases. Therefore, it would seem likely that, whereas these nucleoside analogues are not phosphorylated in normal uninfected cells, they are effectively phosphorylated in HSV- (or VZV)-infected cells, assuming that K_i is a reflection of the drug affinity for the enzyme and that K_m values reflect its ability to serve as an alternative substrate.

A marked difference was noted in the affinity of *Z*-5-(2-bromovinyl)-dUrd and *E*-5-(2-bromovinyl)-dUrd for HSV-1 dThd kinase, and equally striking were the differences in the affinities of the *E*-5-(2-halogenovinyl)-dUrds for HSV-1 dThd kinase and HSV-2 dThd kinase (Table 2). These differences correlated well with the differential activity of *Z*- and *E*-5-(2-bromovinyl)-dUrd against HSV-1, on the one hand, and the differential susceptibility of HSV-1 and HSV-2 toward *E*-5-(2-halogenovinyl)-2′-deoxyuridines, on the other hand (Table 1). Thus, the antiherpes activity of 5-(2-halogenovinyl)-substituted dUrd analogues is critically dependent on their phosphorylation by the virus-induced dThd kinase. A similar conclusion has been reached previously for various other 5-substituted 2′-deoxyuridines, e.g., 5-ethyl-dUrd, 5-propyl-dUrd, and 5-propynyloxy-dUrd (17, 18), and for a nonpyrimidine nucleoside analogue, 9-(2-hydroxyethoxymethyl)guanine (acycloguanosine) (19).

Our data are consistent with the discriminating behavior of *E*-5-(2-halogenovinyl)-dUrds toward HSV-1 and HSV-2. This discrimination is primarily based upon their differential affinity for the HSV-1 and HSV-2 dThd kinases. Likewise, the superiority of *E*- over *Z*-5-(2-bromovinyl)-dUrd as an anti-HSV-1 agent appears to relate to its affinity for the HSV-1 dThd kinase. The latter observation points to the importance of the steric configuration of the halogenovinyl group in the interaction of the dUrd analogue with HSV-1 dThd kinase. These findings have both theoretical and practical implications. Whereas they may help to resolve the molecular determinants that govern the interaction of HSV-1 dThd kinase with its substrate, they may enable the synthesis of other nucleoside analogues with selective antiherpes activity.

The picture now emerging for the mechanism of antiviral action of *E*-5-(2-bromovinyl)-dUrd (and the other *E*-5-(2-halogenovinyl)-2′-deoxyuridines) is that their selectivity as antiherpes agents would, to a large extent, be determined by their specific affinity for the virus-induced dThd kinase. As a consequence, their phosphorylation to the 5′-triphosphate form would be restricted to the virus-infected cell. After they have been converted to the 5′-triphosphate, they would inhibit the DNA polymerization reaction, thereby inhibiting HSV-1 DNA polymerase to a greater extent than the cellular polymerases α and β (20). It now appears the *E*-5-(2-halogenovinyl)-dUrd may also be incorporated into DNA of HSV-infected cells.[6] To what extent this incorporation contributes to the antiviral activity of the compound remains to be established.

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