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DIFFERENTIAL BINDING AFFINITIES OF SUGAR-MODIFIED DERIVATIVES OF (*E*)-5-(2-BROMOVINYL)-2′-DEOXYURIDINE FOR HERPES SIMPLEX VIRUS-INDUCED AND HUMAN CELLULAR DEOXYTHYMIDINE KINASES*

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Abstract—The affinity of a large number of sugar-modified derivatives of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) was determined towards deoxythymidine (dThd) kinases (TK) of various origin, i.e. human cytosol and mitochondrial TK, as well as herpes simplex virus (HSV) type 1 and type 2 TK. Substitution at the 3′- and 5′-position had differential effects on the interaction of BVDU with TK from different sources. The binding affinity of the nucleoside analogs for these different TKs was also influenced by the nature of the 5-substituent (2-bromovinyl vs 2-chlorovinyl). The 5′-azido and 5′-amino derivatives of BVDU showed affinity for HSV-1 TK only and may, therefore, be useful to differentiate HSV-1 TK from all other TKs. There was no stringent correlation between the antiviral effects of the compounds and their binding constants for viral TK, suggesting that phosphorylation by viral TK is an essential but not sufficient factor in determining the antiviral activity of these analogs.

The deoxythymidine kinases (TK) induced by herpes simplex virus (HSV) and varicella zoster virus (VZV) have been recognized as potential targets in the development of selective antiviral compounds. The strategy thereby followed is based on the "specific alternative substrate" approach or differential substrate specificity of virus-induced TK versus host cellular TK [1]. Several nucleoside analogs have been found to exert their selectivity as antiviral agents primarily because of their preferential substrate affinity for virus TK [2–11]. HSV-1, HSV-2 and VZV each induce a unique TK with different substrate specificity than the host, for example, HSV-2 TK shows less tolerance toward dThd analogs with substitution at the 5-position than does HSV-1 TK. Recently, several analogs of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) [12] with modifications at the 3′- or 5′-position of the sugar moiety were synthesized [13] and found to have varying degrees of anti-HSV-1 and HSV-2 activities [13, 14]. These analogs (Fig. 1) have been used to establish the structural determinants of the dThd analogs involved in their interaction with TK and to further elucidate the active binding site of TK from HSV-1, HSV-2, human cytosol and mitochondria.

MATERIALS AND METHODS

Chemicals. dThd was obtained from the Sigma Chemical Co., St. Louis, MO; [2-^{14}C]dThd and [γ-^{32}P]ATP were purchased from the New England Nuclear Corp. Boston, MA. All other chemicals were of reagent grade or better.

Sugar-modified BVDU and CVDU. The sugar-modified derivatives of BVDU and its 5-(2-chlorovinyl) counterpart, CVDU, were synthesized by R. Busson, L. Colla and H. Vanderhaeghe (Rega Institute, University of Leuven, Belgium) according to previously reported procedures [13].

Enzyme preparations. The cellular cytosol and mitochondrial dThd kinases were isolated from 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 HSV-1 (strain KOS) and HSV-2 (strain 333) respectively. The enzymes were purified with affinity chromatography on a column of *p*-aminophenyl-thymidine-3′-phosphate-Sepharose [15]. When needed, the enzyme was concentrated on a DEAE-cellulose column (0.5 × 4.0 cm); the loading buffer consisted of 50 mM Tris–Cl (pH 8.8), 2 mM dithiothreitol, 1 mM EDTA, 0.01 mM dThd, 20% glycerin; the eluting buffer was 300 mM Tris–Cl (pH 6.8), 2 mM dithiothreitol, 1 mM EDTA, 0.01 mM dThd, 20% glycerin and 0.1% bovine serum albumin.

Enzyme assay. The assay procedure was the same as that described previously [15]. Incubation was conducted at 37° for 30 min. The K_i values were determined as described [16].

Assay for phosphorylation. The procedure of Dobersen and Greer [17] was applied to test the ability of the sugar-modified BVDU and CVDU derivatives to act as alternative substrates of HSV-1 and HSV-2 kinases. The concentrations of [γ-^{32}P]ATP and the sugar-modified derivatives were 0.5 and 0.2 mM respectively.

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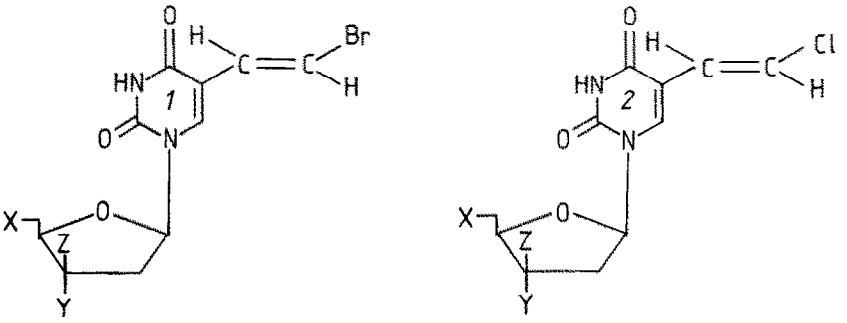

NUCLEOSIDE	BASE	X	Y	Z
BVDU	1	OH	OH	H
5'-Cl-BVDU	1	Cl	OH	H
5'-Br-BVDU	1	Br	OH	H
5'-I-BVDU	1	I	OH	H
5'-N_3-BVDU	1	N_3	OH	H
5'-NH_2-BVDU	1	NH_2	OH	H
BVxyloDU	1	OH	H	OH
3'-Cl-BVDU	1	OH	Cl	H
3'-NH_2-BVDU	1	OH	NH_2	H
3'-N_3-BVxyloDU	1	OH	H	N_3
CVDU	2	OH	OH	H
5'-N_3-CVDU	2	N_3	OH	H
3'-NH_2-CVDU	2	OH	NH_2	H

Fig. 1. Structural formulae of BVDU and CVDU derivatives.

Table 1. Inhibition constants of BVDU derivatives for various kinds of dThd kinase*

	K_i(μM)			
Compound	Cytosol	Human Mitochondrial	HSV-1 (strain KOS)	HSV-2 (strain 333)
BVDU	> 100	0.9	0.4	3.0
5'-Cl-BVDU	> 100	27	1.4	80
5'-Br-BVDU	> 100	—	2.7	30
5'-I-BVDU	> 100	8	3.9	50
5'-N_3-BVDU	> 100	> 100	6.8	> 100
5'-NH_2-BVDU	> 100	> 100	4.2	> 100
3'-Cl-BVDU	> 100	0.9	0.6	23
3'-NH_2-BVDU	> 100	20	1.9	35
BVxyloDU	> 100	53	28.5	> 100
3'-N_3-BVxyloDU	> 100	1.9	108	25
CVDU†	> 100	0.29	0.24	2.0
5'-N_3-CVDU	61	14	4.2	11.3
3'-NH_2-CVDU	52	9	1.1	14.7

* The K_i values were determined by the procedure of Cheng and Prusoff [16]. The amount of enzyme used was 0.02, 0.05, 0.1 and 0.1 units for human cytosol, human mitochondrial, HSV-1 (strain KOS) and HSV-2 (strain 333) dThd kinase respectively. The volume of the reaction mixture was 0.1 ml.

† The data for CVDU were taken from Ref. 9.

Assay for inhibition of virus replication. Inhibition of virus-induced cytopathogenicity in primary rabbit kidney cell cultures was measured following the procedure described by De Clercq *et al.* [18].

RESULTS AND DISCUSSION

The interaction of the nucleoside analogs with TK was examined by estimating the K_i values of each compound for the phosphorylation of [^{14}C]dThd by TK from different sources. The results are summarized in Table 1. All compounds, with the exception of BVxyloDU and 3′-N_3BVxyloDU, were potent inhibitors of HSV-1 TK. This was also the case for the 5′-modified derivatives which are unlikely to be phosphorylated by TK due to the absence of the 5′-hydroxyl group. A less potent inhibition was observed with HSV-2 TK. This was particularly striking for 5′-Cl-BVDU, 5′-NH_2-BVDU and 3′-Cl-BVDU which inhibited HSV-2 TK at a concentration that was 25-fold higher than that required for inhibition of HSV-1 TK. For some compounds, i.e. CVDU, BVDU and 3′-Cl-BVDU, mitochondrial TK was inhibited to a similar extent as HSV-1 TK. However, for several other compounds, i.e. 5′-N_3-BVDU and 5′-NH_2-BVDU, mitochondrial TK did not show any appreciable affinity. As a rule, none of the BVDU derivatives showed any binding affinity for the cytosol TK (Table 1).

The inhibitory potency of the BVDU analogs towards HSV-1 TK is primarily determined by the presence of a bromovinyl group at the C-5 position of the uracil ring. Substitution of 5′-OH of BVDU or CVDU by an amino or azido group has different effects as far as the interaction of those analogs with various types of TK is concerned. In comparison with CVDU, 5′-N_3-CVDU has a somewhat greater binding affinity for human cytosol TK but binds 50-fold less efficiently to human mitochondrial TK, 16-fold less efficiently to HSV-1 TK, and 5-fold less efficiently to HSV-2 TK. This suggests that the structural arrangement of the binding sites of 5′-OH of CVDU for various types of TK may be different.

The binding site of the nucleoside C-5′ for HSV-2 TK and mitochondrial TK, but not for HSV-1 TK, may be influenced by the nature of the C-5 substituent. Indeed, 5′-N_3-BVDU does not exhibit any binding affinity towards mitochondrial TK and HSV-2 TK ($K_i > 100\ \mu$m), whereas 5′-N_3-CVDU is a relatively good inhibitor of both the mitochondrial and HSV-2 enzyme ($K_i \sim 10\ \mu$M). Unlike the 5′-amino derivatives of dThd and IDU (5-iodo-2′-deoxyuridine) [11], 5′-NH_2-BVDU is a poor inhibitor of mitochondrial TK and HSV-2 TK, with a $K_i > 100\ \mu$M. However, 5′-NH_2-BVDU is a good inhibitor of HSV-1 TK. It is likely that 5′-NH_2-BVDU, like 5′-NH_2-IDU and 5′-NH_2-dThd, could be phosphorylated by HSV-1 TK. Since HSV-1 TK is the only one of a variety of dThd kinases tested which exhibited a binding affinity for 5′-NH_2-BVDU and 5′-N_3-BVDU, these compounds should be considered as tools to differentiate HSV-1 TK from the other TK types.

The binding affinities of BVDU and CVDU for the different TKs were also influenced by modifications at the 3′-position, i.e. substitution of a chlorine for 3′-OH of BVDU did not alter its binding affinity to mitochondrial TK or HSV-1 TK, but markedly decreased its binding affinity to HSV-2 TK. The K_i values for the two xylo derivatives also varied depending on the source of TK. This further supports the concept that these TKs differ in terms of catalytic binding site.

Since the 3′-substituted derivatives of BVDU or CVDU can serve as substrate for phosphorylation by TK, their phosphorylation rate relative to that of dThd was examined with both HSV-1 TK and HSV-2 TK, and the results are presented in Table 2. Substitution of the 3′-hydroxyl group by a chlorine or amino group decreased the phosphorylation rate of BVDU and CVDU, and the xylo derivative was totally inert as substrate for the viral TK.

While most 3′- and 5′-substituted BVDU and CVDU analogs showed little, if any, antiviral activity, some compounds, i.e. 5′-I-BVDU, 3′-NH_2-BVDU and 3′-NH_2-CVDU, exhibited a marked activity against HSV-1 (Table 3). These compounds were, like BVDU and CVDU themselves, much less active against HSV-2. The selective anti-HSV-1 activity of 5′-I-BVDU, 3′-NH_2-BVDU and 3′-NH_2-CVDU may, akin to that of BVDU, CVDU [9] and other antiviral nucleoside analogs [10], depend on a specific phosphorylation by the HSV-1-encoded dThd kinase, since all these nucleoside analogs

Table 2. Relative phosphorylation rates of BVDU derivatives by HSV-1 and HSV-2 dThd kinase

Compound (0.02 mM)	Velocity (%) relative to dThd*	
	HSV-1 (strain KOS)	HSV-2 (strain 333)
dThd	100	100
BVDU	109	115
3′-Cl-BVDU	40	<3
3′-NH_2-BVDU	48	20
BVxyloDU	<3	<3
CVDU†	96	112
3′-NH_2-CVDU	39	21

* The amount of enzyme used and the volume of the reaction mixture are the same as indicated in the footnote of Table 3.

† The data for CVDU were taken from Ref 9.

Table 3. Inhibitory effects of BVDU derivatives on HSV-1 and HSV-2 replication in primary rabbit kidney cell cultures

Compound	Minimal inhibitory concentration* (μg/ml) HSV-1	HSV-2
BVDU	0.008	1
5′-Cl-BVDU	≥ 100	≥ 200
5′-Br-BVDU	100	100
5′-I-BVDU	1	10
5′-N_3-BVDU	> 200	≥ 200
5′-NH_2-BVDU	40	> 200
BVxyloDU	> 200	> 200
3′-Cl-BVDU	> 200	> 200
3′-NH_2-BVDU	0.1	25
3′-N_3-BVxyloDU	200	150
CVDU	0.02	2
5′-N_3-CVDU	200	200
3′-NH_2-CVDU	0.2	80

* Concentration of compound required to reduce virus-induced cytopathogenicity by 50%. Average values for three laboratory strains (KOS, F and McIntyre) of HSV-1 and three laboratory strains (Lyons, G and 196) of HSV-2 are presented.

showed a much lower K_i, thus higher binding affinity, for HSV-1 dThd kinase than for the host cell (cytosol) dThd kinase (Table 1).

On the other hand, the differences shown in anti-HSV-1 activity by the 3′- and 5′-substituted BVDU derivatives (Table 3) did not closely correlate with their respective binding affinities for HSV-1 TK (Table 1). In fact, some BVDU derivatives with a strong binding affinity for HSV-1 dThd kinase, i.e. 5′-Cl-BVDU and 3′-Cl-BVDu, were less active as antiviral agents than BVDU derivatives with a lower affinity for dThd kinase, i.e. 5′-I-BVDU and 3′-NH_2-BVDU. This lack of correlation suggests that, besides phosphorylation by the virus-induced dThd kinase, other factors may contribute to the selective antiviral action of the BVDU analogs, viz. interaction with the viral DNA polymerase or incorporation into DNA. Thus, phosphorylation by the viral TK may be an essential but not sufficient factor in determining the antiviral activity of these nucleoside analogs.

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