Antiviral Research, 8 (1987) 41–51
Elsevier

AVR 00252

Differential metabolism of (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (IVDU) by equine herpesvirus type 1- and herpes simplex virus-infected cells

Saul Kit[1], Hiroshi Ichimura[1] and Erik De Clercq[2]

[1]*Division of Biochemical Virology, Baylor College of Medicine, Houston, U.S.A.;* [2]*Rega Institute, Katholieke Universiteit Leuven, Leuven, Belgium*

(Received 13 March 1987; accepted 26 May 1987)

Summary

Thymidine kinase (TK) enzymes encoded by herpes simplex viruses types 1 and 2 (HSV-1, HSV-2), and equine herpesvirus type 1 (EHV-1) catalyze the phosphorylation of thymidine (dThd) and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU). The replication of HSV-1 is sensitive to BVDU, but the replication of HSV-2 and EHV-1 is not. To investigate the differential sensitivity of the viruses to halogenated vinyldeoxyuridine drugs, the phosphorylation of ^{125}I-labeled (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (IVDU) was studied. Cytosol enzymes from cells infected by HSV-2 and EHV-1 phosphorylated [^{125}I]IVDU to the monophosphate, IVDUMP, but did not convert IVDUMP to higher di- plus triphosphates (IVDUDP plus IVDUTP) forms. In contrast, enzymes from HSV-1-infected cells converted [^{125}I]IVDU to radioactive IVDUMP and IVDUDP plus IVDUTP. Experiments with mixtures of EHV-1- and HSV-1-induced enzymes showed that the EHV-1 enzyme did not inhibit formation of the IVDUDP plus IVDUTP by the HSV-1 enzyme. With [^{125}I]IVDU as substrate, the K_m values for the EHV-1 and HSV-1 TKs were 1.82 and 0.34 μM, respectively, and the K_i (dThd) value for the EHV-1 TK was 0.35 μM. In vivo experiments showed that HSV-1-infected cells converted IVDU to the mono- and the di- plus triphosphate forms. In contrast, EHV-1-infected cells converted IVDU to the monosphate to a lesser extent than did HSV-1-infected cells, and did not produce the di- plus triphosphates. Thus, inefficient

Abbreviations: tk=thymidine kinase gene; TK= thymidine kinase activity or polypeptide.

Correspondence to: Dr. Saul Kit, Division of Biochemical Virology, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030, U.S.A.

phosphorylation of the monophosphates probably contributes to the insensitivity of EHV-1 replication to IVDU, as it does to the insensitivity of HSV-2 replication to this drug.

Thymidine kinases of herpes viruses; Phosphorylation of iodovinyl deoxyuridine (IVDU); K_m for [^{125}I] IVDU of thymidine kinases; IVDU triphosphate synthesis; Herpes inhibition by IVDU

Introduction

(*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) is a potent and selective antiherpes agent (Allaudeen et al., 1981; De Clercq, 1982; De Clercq, 1984). The selective antiherpes activity of BVDU depends, in part, on a specific phosphorylation by a virus-induced thymidine–thymidylate kinase (Cheng et al., 1981; De Clercq, 1982; Fyfe, 1982; Fyfe et al., 1983). In its triphosphate form, BVDU specifically inhibits viral DNA polymerase activity (Allaudeen et al., 1981; Ruth and Cheng, 1981), and can be incorporated into viral DNA. This incorporation changes the stability and/or function of the DNA (Mancini et al., 1983; Prusoff et al., 1984). In addition, a phosphorylated derivative of BVDU can interfere with glycosylation of virus proteins (Olofsson et al., 1985; Siegel et al., 1984).

BVDU is about 150–200 times less inhibitory for herpes simplex virus type 2 (HSV-2) than for HSV-1 (Cheng et al., 1981). This differential sensitivity of HSV-1 and HSV-2 to BVDU has been partially explained by the failure of HSV-2-infected cells to convert the 5′-monophosphate of BVDU to the 5′-diphosphate and the 5′-triphosphate (Ayisi et al., 1984; Descamps and De Clercq, 1981; Fyfe, 1982; Fyfe et al., 1983).

Equine herpesvirus type 1 (EHV-1) is relatively resistant to BVDU (De Clercq, 1982; Kit et al., 1987). Nevertheless, EHV-1 does induce a virus-encoded thymidine kinase (TK) (Allen et al., 1978; Allen et al., 1979), and BVDU is a competitive inhibitor of deoxythymidine (dThd) phosphorylation by the EHV-1-induced TK (Kit et al., 1987). Hence, it seemed possible that the insensitivity of EHV-1 replication to BVDU was, at least in part, attributable to the failure of EHV-1-infected cells to phosphorylate the monophosphate form of BVDU to the di- and triphosphate. The analog of BVDU, that is, (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (IVDU) is another 5-substituted 2′-deoxyuridine which shows HSV-1 specificity (De Clercq, 1982; De Clercq, 1984; De Clercq et al., 1980; Descamps and De Clercq, 1981). Since the radiolabeled IVDU was available, it was of interest to compare the phosphorylation of (*E*)-5-(2-^{125}I-vinyl)-2′-deoxyuridine ([^{125}I]IVDU) by cytosol extracts prepared from EHV-1-, HSV-1-, HSV-2-, and pseudo-rabies virus (PRV)-infected cells (De Clercq et al., 1980; De Clercq et al., 1981; Descamps and De Clercq, 1981). In addition, the in vivo metabolism of [^{125}I]IVDU in mock-, EHV-1-, and HSV-1-infected cells was studied.

Materials and Methods

Cells

Equine dermis (Eq.De.), rabbit skin (RAB-9), African green monkey kidney (Vero), and owl monkey kidney (OMK) cells were grown in Auto Pow Eagle's minimal essential medium (APMEM, Flow Laboratories, McLean, VA) supplemented with 10% bovine fetal serum (BFS), 2 mM glutamine, 0.005% neomycin, 20 mM bicarbonate, and 10 mM Hepes buffer (pH 7.3). Bromodeoxyuridine (BrdUrd)-resistant, tk-negative (tk^-) mutant, RAB(BU) cells were grown in the same medium as that of the parental RAB-9 cells, but were supplemented with BrdUrd (25 μg/ml) (Sigma Chemical Co., St. Louis, MO), except for the passage preceding each experiment (Kit and Qavi, 1983).

Viruses

Equine rhinopneumonitis [equine herpesvirus type 1 (EHV-1)] strain (RQ) is an EHV-1 subtype-1 strain that had been adapted to grow at 26°C in Vero cells (Purdy et al., 1977). Stocks of EHV-1(RQ) were prepared by infecting Vero cells at about 0.01 pfu/cell and incubating the infected culture for 2–3 days at 34.5°C (Kit et al., 1987). The titers of the virus harvests were about 10^8–10^9 pfu/ml. Stocks of HSV-1(KOS), HSV-2(333), and PRV(BUK-5) were prepared in OMK and RAB-9 cells, respectively, as described (Kit and Dubbs, 1977; Kit et al., 1985).

Compounds

The nonlabeled compounds, (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (IVDU), IVDU-5′-monophosphate (IVDUMP), and IVDU-3′,5′-diphosphate (IVDUDP) were synthesized at the Rega Institute. Radioactive (*E*)-5-(2-^{125}I-vinyl)-2′-deoxyuridine (18.2–31.0 mCi/μmol) was synthesized by Dr. A. Verbruggen (Department of Nuclear Medicine-Radiopharmacy, University Hospital Gasthuisberg, B-3000 Leuven, Belgium) (De Clercq et al., 1980).

Enzyme assays

Cytosol extracts were prepared from mock-infected and from virus-infected cells, and assayed for TK activity as described previously (Dubbs and Kit, 1964; Kit et al., 1974), except that [^{125}I]IVDU, 1.1 μM, and 4.5–8.9 × 10^3 counts per minute (cpm) per picomole, was used as the nucleoside substrate. The reaction products were separated from the [^{125}I]IVDU by thin-layer chromatography (TLC) on PEI-cellulose F precoated TLC plastic sheets (EM Laboratories, Elmsford, NY) with 0.5 M sodium formate (pH 3.4) as the solvent (RF values: IVDUMP [0.39] and IVDUDP [0.06]) (Kit and Kit, 1975). Nonradioactive IVDUMP and IVDU-3′,5′-diphosphate were used as markers. In this system, IVDUDP was not clearly separated from IVDU-5′-triphosphate (IVDUTP), which remained close to the ori-

gin. Therefore, the radioactive spots corresponding to IVDUMP, and the spot corresponding to the diphosphate combined with the spot corresponding to the IVDU-5′-triphosphate (IVDUTP), were cut out and counted with a Packard Tri-Carb liquid scintillation spectrometer (Kit et al., 1966). The virus-induced TKs were also partially purified by ammonium sulfate precipitation (Kit et al., 1986; Kit et al., 1987) and used for enzyme kinetic studies.

Phosphorylation of [^{125}I]IVDU in vivo

The in vivo experiments were modified from the methods described by Descamps and De Clercq (Descamps and De Clercq, 1981). Subconfluent RAB(BU) or Eq.De. cell monolayer in 2-oz prescription bottles (Sani-Glas, Brockway Glass Co., Inc.) (approximately 1.5×10^6 cells/bottle) was either mock-infected or infected with EHV-1(RQ) or HSV-1(KOS) at 20 and 1 pfu per cell, respectively. After a 1-h incubation at 37°C, the inoculum was removed and 3 ml of APMEM with 10% BFS were added to each bottle. At 2 h before the harvest time, the medium was taken off and 2 ml of APMEM with 10% FBS containing [^{125}I]IVDU (10 or 100 μCi per ml and 0.55 or 5.5 μM, respectively) were added to each bottle. At 8 h (HSV-1) or 16 h (mock, EHV-1) postinfection, the labeling medium was removed, the cells were washed 3 times with cold phosphate buffered saline, and 0.5 ml of 10% trichloroacetic acid (TCA) was added. After a 10-min incubation in an ice bath, the cells were frozen at −80°C and thawed, and the TCA-insoluble fractions were separated from the TCA-soluble fractions by centrifugation at 13000 rpm at 4°C for 15 min in a Sorvall centrifuge. TCA-soluble fractions (20 μl) were applied to the TLC sheet, chromatographed, and counted as described above.

Plaque autoradiography

The plaque autoradiographic experiments were done as described previously (Kit et al., 1985; Siegel et al., 1984), except for using either 3 μCi of [2-^{14}C]thymidine (57 mCi/mmol) (ICN Pharmaceuticals, Inc., Irvine, CA) or 3 μCi of [^{125}I]IVDU (31 mCi/μmol) to label the virus DNA.

Results

Phosphorylation of [^{125}I]IVDU by cytosol extracts from virus-infected cells

To investigate whether the EHV-1-induced TK catalyzes the phosphorylation of IVDU to IVDUMP and to higher phosphate forms, cytosol extracts were prepared from mock-infected and virus-infected TK-negative, RAB(BU) cells and assayed for [^{125}I]IVDU-phosphorylating activity (Table 1). For each of the virus infections, the time after infection for the preparation of enzyme extracts corresponded to the time of maximal TK induction. Cytosol extracts from HSV-1-infected RAB(BU) cells not only catalyzed the phosphorylation of IVDU, but

TABLE 1

Phosphorylation of [^{125}I]IVDU by cytosol extracts from virus-infected tk$^-$ RAB(BU) cells.

Experiment	Source of enzyme[a]	Time of enzyme assay (min)	Phosphorylation of [^{125}I]IVDU to:	
			IVDUMP	IVDUDP + IVDUTP
			(cpm/μg protein)	
1	Mock-infected	10	30	3
	HSV-1-infected		1,470	170
	EHV-1-infected		610	1
2	Mock-infected	30	195	1
	HSV-1-infected		1,390	560
	EHV-1-infected		1,340	2
3	Mock-infected	30	251	1
	HSV-1-infected		1,330	400
	HSV-2-infected		1,780	2
	EHV-1-infected		1,280	3
	PRV-infected		1,750	50
4	Mock-infected	30	211	1
	PRV-infected		2,090	70

[a] Cytosol extracts were prepared from HSV-1-, HSV-2-, and PRV-infected cells at 7 h postinfection, and from EHV-1- and mock-infected cells at 16 h postinfection.

converted the IVDUMP to IVDUDP plus IVDUTP. In contrast, extracts from EHV-1- and HSV-2-infected RAB(BU) cells catalyzed the phosphorylation of IVDU, but did not convert the IVDUMP to IVDUDP plus IVDUTP. The PRV-induced TK also catalyzed the phosphorylation of IVDU and converted the IVDUMP to IVDUDP plus IVDUTP, but the phosphorylation rate of IVDUMP of the PRV-induced enzyme was not so high as that of the HSV-1-induced enzyme.

To learn whether the inability of the extracts from EHV-1-infected cells to convert IVDU to IVDUDP + IVDUTP was attributable to an inhibitor in the extracts from EHV-1-infected cells, e.g., nucleoside pyrophosphatase activities, mixtures of cytosol extracts from HSV-1- and EHV-1-infected cells were assayed for

TABLE 2

Phosphorylation of [^{125}I]IVDU by mixtures of cytosol extracts from HSV-1- and EHV-1-infected cells[a].

Virus-specific enzyme	Vol. of enzyme used in assay (μl)	cpm IVDUMP	cpm IVDUDP + IVDUTP
HSV-1	20[b]	30,380	2,950
HSV-1	40	28,000	4,530
EHV-1	20[c]	7,730	14
EHV-1	40	13,500	49
EHV-1 plus	20		
HSV-1	20	30,400	2,510

[a] Extracts were incubated for 30 min at 38°C.

[b] 20 μl contains 95 μg protein.

[c] 20 μl contains 107 μg protein.

IVDU-phosphorylating activity (Table 2). The phosphorylation of IVDU to IVDUMP and to IVDUDP + IVDUTP by cytosol extracts from HSV-1-infected cells in the presence of cytosol extracts from EHV-1-infected cells was about 85% of that in the absence of the extracts from EHV-1-infected cells, contraindicating the presence of powerful inhibitors of phosphorylation in the extracts from EHV-1-infected cells.

Enzyme kinetic studies using [^{125}I]IVDU as a substrate

Kinetic studies to determine the K_m values of IVDU for the EHV-1- and HSV-1-induced IVDU-phosphorylating enzymes and K_i value of dThd for the EHV-1-induced enzyme are presented in Fig. 1. IVDU was a good substrate for the EHV-1-induced TK, although the HSV-1-induced TK had a higher affinity for IVDU (K_m = 0.34 μM) than did the EHV-1-induced TK (K_m = 1.82 μM). dThd was a

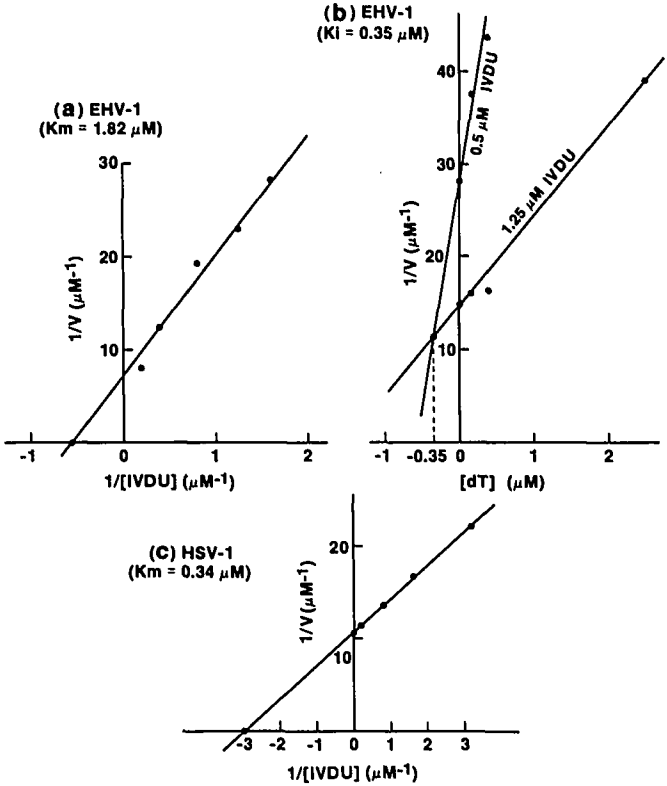

Fig. 1. Lineweaver–Burk plots for the determination of the K_m values (IVDU) for partially purified (a) EHV-1-induced and (c) HSV-1-induced enzymes, and Dixon plot (b) showing the inhibition by dThd of the IVDU phosphorylation reaction catalyzed by the EHV-1-induced enzyme. Initial reaction rates were measured by incubating different concentrations of [^{125}I]IVDU with [(a), (c)] or without (b) dThd in the presence of ATP (8 mM) and Mg^{2+} (4 mM) for 3 min at 38°C.

TABLE 3

Kinetic constants of nucleosides as substrates and inhibitors of partially purified TK activities from HSV-1- and EHV-1-infected RAB(BU) cells.

Herpesvirus TK	[^{3}H]dThd[a]		[^{125}I]IVDU	
	K_m (dThd)	K_i (BVDU)	K_m (IVDU)	K_i (dThd)
HSV-1	0.32[b]	0.09	0.34	Not done
EHV-1	0.58	1.35	1.82	0.35

[a] Experiments utilizing [^{3}H]dThd as substrate are from Kit et al. (1987).
[b] Kinetic constants are expressed as μM.

strong inhibitor of IVDU phosphorylation by the EHV-1-induced enzyme (K_i = 0.35 μM). These results complement our previous study showing that, with [^{3}H]dThd as a substrate, the K_i (BVDU) value for the EHV-1-induced TK was about 1.35 μM, while the K_i (BVDU) value for the HSV-1-induced TK was 0.09 μM (Kit et al., 1987) (Table 3).

Phosphorylation of [^{125}I]IVDU in vivo

The phosphorylation of [^{125}I]IVDU in EHV-1-infected RAB(BU) cells was assayed and compared with that of the HSV-1-infected cells (Table 4). A significant amount of IVDUMP was detected in the EHV-1-infected RAB(BU) cells only when [^{125}I]IVDU was used at the concentration of 100 μCi/ml and 5.5 μM. The IVDUDP and IVDUTP formed in EHV-1-infected cells at both concentrations of [^{125}I]IVDU were not greater than those formed in mock-infected cells. In contrast, not only IVDUMP but also IVDUDP plus IVDUTP were detected in the HSV-

TABLE 4

Specific phosphorylation of (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (IVDU) by EHV-1- and HSV-1-infected RAB(BU) cells.

Source of extract[a]	Concentration of IVDU[b]	IVDUMP	IVDUDP + IVDUTP
Mock	0.55	92[c]	71
	5.5	91	196
EHV-1	0.55	102	83
	5.5	504	232
HSV-1	0.55	11,230	6,768

[a] RAB(BU) cells were mock-infected or infected with EHV-1 at 20 pfu/cell and with HSV-1 at 1 pfu/cell, and harvested at 16, 16, and 8 h postinfection, respectively.
[b] [^{125}I]IVDU was added at 2 h before each harvest time to the concentration of 10 or 100 μCi/ml, and 0.55 or 5.5 μM, respectively.
[c] Expressed as cpm [^{125}I]IVDUMP or [^{125}I]IVDUDP + [^{125}I]IVDUTP formed in mock- or virus-infected RAB(BU) cells during labeling time per 20 μl out of 500 μl acid-soluble fraction. IVDUMP = IVDU-5′-monophosphate; IVDUDP = IVDU-5′-diphosphate; IVDUTP=IVDU-5′-triphosphate.

1-infected cells at the lower concentration (10 μCi/ml, 0.55 μM) of [^{125}I]IVDU. Similar results were obtained using Eq. De. cells, which are more sensitive hosts for EHV-1 than RAB(BU) cells.

Plaque autoradiography

To investigate whether IVDU was incorporated into EHV-1 DNA, plaque autoradiography experiments using [^{125}I]IVDU or [^{14}C]dThd were carried out. HSV-1-infected cells incorporated [^{125}I]IVDU as well as [^{14}C]dThd into the viral DNA. EHV-1-infected RAB(BU) cells incorporated [^{14}C]dThd, but not [^{125}I]IVDU, into viral DNA (data not shown).

Discussion

The (*E*)-5-(2-halogenovinyl)-2′-deoxyuridine compounds, BVDU and IVDU, are among the most potent and selective inhibitors of HSV-1 replication (De Clercq et al., 1980). However, BVDU and IVDU are much less inhibitory for EHV-1 than for HSV-1, and 100–200 times less inhibitory for HSV-2 than for HSV-1 (De Clercq, 1982, 1984; De Clercq et al., 1980). Using [^{125}I]IVDU as a probe, we investigated the biochemical mechanism(s) involved in the inactivity of the (*E*)-5-(2-halogenovinyl)-2′-deoxyuridine against EHV-1 replication.

The in vitro study showed the following: (i) IVDU had a lower affinity for the EHV-1-induced TK (K_m = 1.82 μM) than for the HSV-1-induced TK (K_m = 0.34 μM); (ii) cytosol extracts from EHV-1- and HSV-2-infected TK$^-$ RAB(BU) cells catalyzed the phosphorylation of IVDU but did not convert it to the di- plus triphosphate, whereas extracts from HSV-1-infected cells catalyzed the phosphorylation of IVDU up to the di- plus triphosphates; and (iii) EHV-1 did not induce a strong nucleotide-dephosphorylating activity. These observations indicate that the insensitivity of EHV-1 replication to IVDU, like that of HSV-2, depends, at least in part, on the failure of EHV-1 to phosphorylate IVDU beyond the 5′-monophosphate level (Ayisi et al., 1984; Cheng et al., 1981; De Clercq, 1982; Descamps and De Clercq, 1981; Fyfe, 1982; Fyfe et al., 1983).

The in vivo study showed the following: (i) the EHV-1-infected cells catalyzed the phosphorylation of IVDU, although the phosphorylation rate of IVDU in the EHV-1-infected cells was much lower than in the HSV-1-infected cells, and did not significantly convert it to the di- plus triphosphate; and (ii) the EHV-1-infected cells did not incorporate IVDU into the viral DNA under the same conditions as the HSV-1-infected cells did. These results support the conclusion from the in vitro study.

BVDU and IVDU are active against HSV-1 and PRV, but not against HSV-2 and EHV-1 (De Clercq, 1984). The HSV-1- and PRV-induced TKs catalyzed the phosphorylation of IVDUMP to higher triphosphate forms, although the HSV-1-induced enzymes were more efficient than the PRV-induced enzymes in this regard. In contrast, the HSV-2- and EHV-1-induced TKs did not significantly gen-

erate higher phosphorylated forms of IVDU. It may, therefore, be suggested that not only type specificity but also virus specificity of (*E*)-5-(2-halogenovinyl)-2′-deoxyuridine are determined mainly by conversion of the monophosphate to the diphosphate and subsequently to the triphosphate.

The thymidine kinase activity encoded by HSV-1 is a bifunctional enzyme with an associated thymidylate kinase activity. Previous studies have shown that the thymidine-thymidylate kinase specified by HSV-1 also catalyzed the phosphorylation of BVDUMP to BVDUDP (Fyfe, 1982). Although the virus-coded TK purified from HSV-2-infected cells also phosphorylated dTMP, it did not phosphorylate BVDUMP. Also, HSV-1-coded thymidine kinases purified from cells infected with certain BVDU-resistant HSV-1 mutants catalyzed the phosphorylation of thymidine and BVDU efficiently, but catalyzed the phosphorylation of dTMP at only 2% of the efficiency of the parental enzyme and did not detectably catalyze the phosphorylation of BVDUMP at all (Fyfe et al., 1983; Veerisetty and Gentry, 1985). This selectivity at the second step of BVDUMP phosphorylation correlated well with the effectiveness of the analogue to inhibit HSV replication in cell culture. Alterations in both the thymidine and thymidylate kinase affinities for their substrates and analogues may determine sensitivity to antiviral drugs. In summary, the insensitivity of EHV-1 replication to the (*E*)-5′-(2-halogenovinyl)-2′-deoxyuridine compounds may depend, at least in part, on the absence of a dTMP kinase activity from the EHV-1-encoded TK or the presence of a dTMP kinase activity with poor affinity for BVDUMP and IVDUMP, as has been found with the HSV-2-encoded TK.

Acknowledgements

This investigation was aided by USPHS Research Career Award K6-AI-2352-23 to S.K. and by a research contract from NovaGene, Ltd., Houston, TX. (*E*)-5-(2-iodovinyl)-2′-deoxyuridine (IVDU) was synthesized as described by E. De Clercq and R.T. Walker (1984).

References

Allaudeen, H.S., Kozarich, J.W., Bertino, J.R. and De Clercq, E. (1981) On the mechanism of selective inhibition of herpesvirus replication by (*E*)-5-(2-bromovinyl)-2′-deoxyuridine. Proc. Natl. Acad. Sci. U.S.A. 78, 2698–2702.

Allen, G.P., McGowan, J.J., Bryans, J.T., Randall, C.C. and Gentry, G.A. (1978) Induction of deoxythymidine kinase activity in several mammalian cell lines after infection with six different strains of equine herpesvirus type 1 (EHV-1). Virology 90, 351–359.

Allen, G.P., McGowan, J.J., Randall, C.C., Mancini, W., Cheng, Y.-C. and Gentry, G.A. (1979) Purification and characterization of deoxythymidine kinase (dTK) induced in dTK$^-$ 3T3 mouse cells by equine herpesvirus type 1 (EHV-1). Virology 92, 367–374.

Ayisi, N.K., De Clercq, E., Wall, R.A., Hughes, H. and Sacks, S.L. (1984) Metabolic fate of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine in herpes simplex virus- and mock-infected cells. Antimicrobiol. Agents Chemother. 26, 762–765.

Cheng, Y.-C., Dutschman, G., De Clercq, E., Jones, A.S., Rahim, S.G., Verhelst, G. and Walker, R.T. (1981) Differential affinities of 5-(2-halogenovinyl)-2′-deoxyuridine for deoxythymidine kinases of various origins. Mol. Pharmacol. 20, 230–233.

De Clercq, E. (1982) Selective antiherpes agents. Trends Pharmacol. Sci. 3, 492–495.

De Clercq, E. (1984) The antiviral spectrum of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine. J. Antimicrob. Chemother. 14 (Suppl. A), 85–95.

De Clercq, E. and Walker, R.T. (1984) Synthesis and antiviral properties of vinylpyrimidine nucleoside analogs. Pharmacol. Ther. 26, 1–44.

De Clercq, E., Descamps, J., Verhelst, G., Walker, R.T., Jones, A.S., Torrence, P.F. and Shugar, D. (1980) Comparative efficacy of antiherpes drugs against different strains of herpes simplex virus. J. Infect. Dis. 141, 563–574.

De Clercq, E., Heremans, H., Descamps, J., Verhelst, G., De Ley, M. and Billiau, A. (1981) Effects of *E*-5-(2-bromovinyl)-2′-deoxyuridine and other selective anti-herpes compounds on the induction of retrovirus particles in mouse BALB/3T3 cells. Mol. Pharmacol. 19, 122–129.

Descamps, J. and De Clercq, E. (1981) Specific phosphorylation of *E*-5-(2-iodovinyl)-2′-deoxyuridine by herpes simplex virus-infected cells. J. Biol. Chem. 256, 5973–5976.

Dubbs, D.R. and Kit, S. (1964) Isolation of properties of vaccinia mutants deficient in thymidine kinase-inducing activity. Virology 22, 214–225.

Fyfe, J.A. (1982) Differential phosphorylation of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine monophosphate by thymidylate kinases from herpes simplex viruses type 1 and 2 and varicella zoster virus. Mol. Pharmacol. 21, 432–437.

Fyfe, J.A., McKee, S.A. and Keller, P.M. (1983) Altered thymidine-thymidylate kinases from strains of herpes simplex virus with modified drug sensitivities to acyclovir and (*E*)-5-(2-bromovinyl)-2′-deoxyuridine. Mol. Pharmacol. 24, 316–333.

Kit, S. and Dubbs, D.R. (1977) Regulation of herpesvirus thymidine kinase activity in LM(TK$^-$) cells transformed by ultraviolet light-irradiated herpes simplex virus. Virology 76, 331–340.

Kit, S. and Kit, G. (1975) Analyses of deoxycytidylate deaminase molecular forms in human-mouse and monkey-mouse somatic cell hybrids. Biochem. Genet. 13, 213–226.

Kit, S. and Qavi, H. (1983) Thymidine kinase (TK) induction after infection of TK-deficient rabbit cell mutants with bovine herpesvirus type-1 (BHV-1). Isolation of TK BHV-1 mutants. Virology 130, 381–389.

Kit, S., de Torres, R.A. and Dubbs, D.R. (1966) Arabinofuranosylcytosine-induced stimulation of thymidine kinase and deoxycytidylic deaminase activities of mammalian cultures. Cancer Res. 26, 1959–1966.

Kit, S., Leung, W.-C., Jorgensen, G.N. and Dubbs, D.R. (1974) Distinctive properties of thymidine kinase isozymes induced by human and avian herpesviruses. Int. J. Cancer 14, 598–610.

Kit, S., Kit, M. and Pirtle, E.C. (1985) Attenuated properties of thymidine kinase-negative deletion mutant of pseudorabies virus. Am. J. Vet. Res. 46, 1359–1367.

Kit, S., Kit, M., Ichimura, H., Crandell, R. and McConnell, S. (1986) Induction of thymidine kinase activity by viruses with group B DNA genomes: bovine cytomegalovirus (bovine herpesvirus 4). Virus Res. 4, 197–212.

Kit, S., Ichimura, H. and De Clercq, E. (1987) Phosphorylation of nucleoside analogs by equine herpesvirus type 1 pyrimidine deoxyribonucleoside kinase. Antiviral Res. 7, 53–67.

Mancini, W.R., De Clercq, E. and Prusoff, W.A. (1983) The relationship between incorporation of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine into herpes simplex virus type 1 DNA with virus infectivity and DNA integrity. J. Biol. Chem. 258, 792–795.

Olofsson, S., Lundstrom, M. and Datema, R. (1985) The antiherpes drug (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU) interferes with formation of N-linked and O-linked oligosaccharides of the herpes simplex virus type 1 glycoprotein C. Virology 147, 201–205.

Prusoff, W.H., Mancini, W.R., Lin, T.S., Lee, J.J., Siegel, S.A. and Otto, M.J. (1984) Physical and biological consequences of incorporation of antiviral agents into virus DNA. Antiviral Res. 4, 303–315.

Purdy, C.W., Ford, S.J. and Grant, W.F. (1977) Equine rhinopneumonitis virus (herpesvirus type 1): attenuation in stable monkey cell line. Am. J. Vet. Res. 38, 1211–1215.

Ruth, J.L. and Cheng, Y.-C. (1981) Nucleoside analogues with clinical potential antivirus chemotherapy. The effect of several thymidine and 2′-deoxycytidine analogue 5′-triphosphates on purified human (α, β) and herpes simplex virus (types 1, 2) DNA polymerases. Mol. Pharmacol. 20, 415–422.

Siegel, S.A., Otto, M.J., De Clercq, E. and Prusoff, W.H. (1984) Effect of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine on synthesis of herpes simplex virus type 1-specific polypeptides. Antimicrobiol. Agents Chemother. 25, 566–570.

Tenser, R.B., Jones, J.C., Ressel, S.J. and Fralish, F.A. (1983) Thymidine plaque autoradiography of thymidine kinase-positive and thymidine kinase-negative herpesviruses. J. Clin. Microbiol. 17, 122–127.

Veerisetty, V. and Gentry, G.A. (1985) HSV1-specific thymidylate kinase activity in infected cells. Intervirology 24, 42–49.