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ANTIVIRAL ACTIVITY OF 5-SUBSTITUTED PYRIMIDINE NUCLEOSIDE ANALOGUES

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Abstract - From a large series of 5-substituted 1-(2-deoxy-β-D-ribofuranosyl)-uracil (dUrd), 1-(2-deoxy-β-D-ribofuranosyl)-cytosine (dCyd) and 1-(β-D-arabinofuranosyl)-uracil (araU) analogues that have been evaluated for their antiviral properties, the (E)-5-(2-bromovinyl)derivatives emerged as the most potent and most selective antiherpes agents. (E)-5-(2-Bromovinyl)-dUrd (BVDU) and its congeners, (E)-5-(2-bromovinyl)-dCyd (BVDC) and (E)-5-(2-bromovinyl)-araU (BVaraU), inhibited the replication of herpes simplex virus type 1 (HSV_1) and varicella-zoster virus (VZV) in cell culture at a concentration which was about 5,000- to 50,000-fold lower than the concentration required to affect normal cell growth or metabolism. For example, BVDU inhibited HSV_1 replication in primary rabbit kidney cells and VZV in human embryonic lung cells at a concentration of 0.007 μg/ml (0.02 μM) and 0.003 μg/ml (0.01 μM), respectively. A series of sugar-modified analogues and 3'-O- or 5'-O-acyl esters of BVDU have been prepared, and some of these derivatives, i.e. 3'-amino-BVDU and 5'-O-aminoacetyl-BVDU, proved almost as active as BVDU itself. Further studies revealed that the activity spectrum of BVDU includes, in addition to HSV_1 and VZV, several other herpesviruses such as pseudorabies virus, bovid herpesvirus type 1, simian varicella virus, herpesvirus saimiri and nuclear polyhedrosis virus. Its antiviral action would depend on a specific phosphorylation by the virus-encoded thymidine kinase, a preferential inhibition of the viral DNA polymerase by the 5'-triphosphate of BVDU (BVDUTP), and, finally, the incorporation of BVDUTP into viral DNA. BVDU has demonstrated high efficacy in several animal model infections, and preliminary clinical studies point to the great promise of BVDU in the topical and systemic (i.e. oral) treatment of HSV_1 and VZV infections in humans.

INTRODUCTION

In view of the close resemblance between the metabolic pathways that underlie virus replication and normal cell growth, it has for a long time been considered too formidable a task to design antiviral agents that would eliminate the virus without affecting normal cell metabolism. This prejudice is not longer tenable now that new compounds have been developed which selectively act against herpesvirus replication without danger for the normal cell. Foremost among these selective antiherpes agents are acyclovir (ACV, acycloguanosine, 9-(2-hydroxyethoxymethyl)guanine) (Ref. 1 & 2), bromovinyldeoxyuridine (BVDU, (E)-5-(2-bromovinyl)-2'-deoxyuridine) (Ref. 3 & 4) and fluoroiodoaracytosine (FIAC, 1-(2-fluoro-2-deoxy-β-D-arabinofuranosyl)-5-iodocytosine) (Ref. 5 & 6). With their advent it has become clear that virus infections can be approached by specific chemotherapeutic means.

ACV, BVDU and FIAC are selective inhibitors of herpes simplex virus (HSV) and varicella-zoster virus (VZV), and their selectivity depends primarily on a specific phosphorylation by the virus-induced deoxythymidine-deoxycytidine (dThd-dCyd) kinase which limits their further action to the virus-infected cell. Thus, ACV, BVDU and FIAC are particularly effective against those herpesviruses that code for a dThd-dCyd kinase which recognizes the drugs as substrate. Viruses that do not induce such dThd-dCyd kinase are, as a rule, not susceptible to the inhibitory effects of ACV, BVDU or FIAC.

It is remarkable that the majority of the antiviral drugs that have been licensed for clinical use or are being considered for clinical use are directed toward herpesviruses (HSV,

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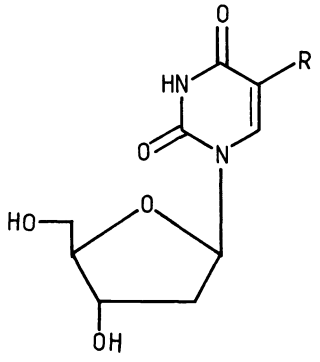
Abbreviations. ID_{50}, inhibitory dose-50; HSV_1, herpes simplex virus type 1; HSV_2, herpes simplex virus type 2; VZV, varicella-zoster virus; PRV, pseudorabies virus; BHV_1, bovid herpesvirus type 1; SVV, simian varicella virus; HVS, herpesvirus saimiri; NPV, nuclear polyhedrosis virus; VV, vaccinia virus; PRK, primary rabbit kidney (cells); FL, feline lung (cells); HEF, human embryo fibroblast (cells); MO, murine fibroblast (cells); Vero, African green monkey kidney (cells); BHK, baby hamster kidney (cells); MDBK, Madin-Darby bovine kidney (cells); OMK, owl monkey kidney (cells); SF, Spodoptera frugiperda (cells).

VZV) and that most of these antiherpes agents are pyrimidine nucleoside analogues. This includes the classical antiherpes drugs idoxuridine (IDU, 5-iodo-2'-deoxyuridine) and trifluridine (TFT, 5-trifluoromethyl-2'-deoxyuridine), which are both used as eye drops for the topical treatment of herpetic keratitis, and the newer antiherpes drugs BVDU and FIAC, which offer great promise for the systemic treatment of HSV and VZV infections. Although structurally related to guanosine, ACV could functionally be regarded as a pyrimidine nucleoside analogue to the extent it is recognized as substrate by the HSV-induced dThd-dCyd kinase (Ref. 7).

In the present report I will review the antiviral activity of a wide variety of 5-substituted pyrimidine nucleoside analogues belonging to either of the following classes : 2'-deoxyuridine (dUrd, 1-(2-deoxy-β-D-ribofuranosyl)-uracil), 2'-deoxycytidine (dCyd, 1-(2-deoxy-β-D-ribofuranosyl)-cytosine) or uracil arabinoside (araU, 1-(β-D-arabinofuranosyl)-uracil). From the whole series of dUrd, dCyd and araU analogues tested, the (E)-5-(2-bromovinyl)derivatives emerged as the most potent and most selective antiherpes agents. BVDU (E)-5-(2-bromovinyl)-dUrd served as the starting material for the synthesis of several sugar-modified analogues and 3'-O- or 5'-O-acyl esters. BVDU has also been the subject of extensive studies aimed at determining its spectrum of antiviral activity and mechanism of action. It has been explored for its efficacy in the treatment of various HSV and VZV infections in animal models and it has recently been submitted to clinical trials in humans.

5-SUBSTITUTED 2'-DEOXYURIDINES

Among the 5-substituted dUrd derivatives (Fig. 1) that were tested for antiviral activity, several compounds, i.e. 5-nitro-dUrd (Ref. 8), 5-ethynyl-dUrd (Ref. 3), 5-ethyl-dUrd (Ref. 9) and 5-propyl-dUrd (Ref. 10), inhibited HSV_1 replication at a relatively low concentration (0.5 - 2 μg/ml) (Table 1) (Ref. 11). Their ID_{50} for HSV_1 was only slightly higher than that of the standard antiherpes drug, 5-iodo-dUrd. These compounds were inhibitory to HSV_2 at similar concentrations as those required to inhibit HSV_1. With the exception of 5-propyl-dUrd, they were also inhibitory to VV. 5-Nitro-dUrd and 5-ethynyl-dUrd interfered with normal cell metabolism (as monitored by incorporation of (1',2'-^{3}H)dUrd or (2-^{14}C)dUrd into DNA) at a concentration which was 10- to 15-fold lower than the minimal antiviral concentration, thus achieving a "negative" selectivity index. These compounds should therefore be considered as cytotoxic rather than antiviral. In contrast with 5-nitro-dUrd and 5-ethynyl-dUrd, the 5-alkyl-2'-deoxyuridines 5-ethyl- and 5-propyl-dUrd proved quite selective in their antiviral activity.



R = I : 5-iodo-dUrd
NO_2 : 5-nitro-dUrd
C≡CH : 5-ethynyl-dUrd
CH_2CH_3 : 5-ethyl-dUrd
$CH_2CH_2CH_3$: 5-propyl-dUrd
$CH=CH_2$: 5-vinyl-dUrd
CH=CHCl(E) : (E)-5-(2-chlorovinyl)-dUrd
CH=CHBr(E) : (E)-5-(2-bromovinyl)-dUrd
CH=CHI(E) : (E)-5-(2-iodovinyl)-dUrd
$CH=CHCH_3$(E) : (E)-5-(1-propenyl)-dUrd
$CH=CHCF_3$(E) : (E)-5-(3,3,3-trifluoro-1-propenyl)-dUrd

Fig. 1. 5-Substituted 1-(2-deoxy-β-D-ribofuranosyl)uracil (2'-deoxyuridine, dUrd) derivatives.

TABLE 1. Antiviral activity of 5-substituted 2'-deoxyuridines

Compound	ID_{50}[a] (µg/ml)			Selectivity index[b]
	HSV_1	HSV_2	VV	
5-Iodo-dUrd	0.13	0.3	0.3	2
5-Nitro-dUrd	2	2	0.2	0.1
5-Ethynyl-dUrd	0.6	1.5	0.2	0.07
5-Ethyl-dUrd	0.5	0.3	1	12
5-Propyl-dUrd	0.6	3	> 200	167
5-Vinyl-dUrd	0.018	0.1	0.4	389
(E)-5-(2-Chlorovinyl)-dUrd	0.02	2	85	3500
(E)-5-(2-Bromovinyl)-dUrd (BVDU)	0.008	1	7	2500
(E)-5-(2-Iodovinyl)-dUrd	0.012	2	10	1667
(E)-5-(1-Propenyl)-dUrd	0.07	22	225	571
(E)-5-(3,3,3-Trifluoro-1-propenyl)-dUrd	0.07	65	350	2143

[a] Dose required to inhibit virus-induced cytopathogenicity in PRK cells by 50 %.
[b] Based on the ratio of ID_{50} for host cell metabolism (dose required to inhibit dUrd incorporation into DNA of uninfected PRK cells by 50 %) to ID_{50} for HSV_1.
Data taken from De Clercq (11). Except for 5-nitro-dUrd, (E)-5-(1-propenyl)-dUrd and (E)-5-(3,3,3-trifluoro-1-propenyl)-dUrd, all ID_{50} values for HSV_1 and HSV_2 represent the average values for 3 laboratory strains and 8 clinical isolates of HSV_1, and 3 laboratory strains and 4 clinical isolates of HSV_2.

The greatest selectivity was demonstrated by 5-vinyl-dUrd (Ref. 3) and the (E)-5-(2-X-vinyl)-dUrd derivatives, (E)-5-(2-chlorovinyl)-dUrd (Ref. 12), (E)-5-(2-bromovinyl)-dUrd (Ref. 3), (E)-5-(2-iodovinyl)-dUrd (Ref. 3), (E)-5-(1-propenyl)-dUrd (Ref. 13) and (E)-5-(3,3,3-trifluoro-1-propenyl)-dUrd (Ref. 13). These compounds were also the most potent inhibitors of HSV_1 replication. Their ID_{50} for HSV_1 ranged from 0.008 to 0.07 µg/ml, that is roughly 3 orders of magnitude lower than the cytotoxic concentration (Table 1). The potent and selective anti-HSV_1 activity of (E)-5-(2-bromovinyl)-dUrd is critically dependent on the E ("Entgegen" or trans) configuration of the 5-substituent, since (Z)-5-(2-bromovinyl)-dUrd, with bromine in the Z ("Zusammen" or cis) position, was about 100 times less active against HSV_1 than the E isomer (Ref. 14).

All (E)-5-(2-X-vinyl)-dUrd derivatives inhibited HSV_2 replication at a concentration that was significantly higher than the concentration required to inhibit HSV_1 replication (Ref. 13) : this difference varied from 100-fold for (E)-5-(2-chlorovinyl)-dUrd to 928 for (E)-5-(3,3,3-trifluoro-1-propenyl)-dUrd. Based on their discriminating behavior toward HSV_1 and HSV_2, the (E)-5-(2-X-vinyl)-dUrd derivatives could be advocated as useful markers for the differentiation between type 1 and type 2 HSV strains in clinical isolates, as originally proposed by De Clercq et al. (4 & 13) and later substantiated by Mayo (15).

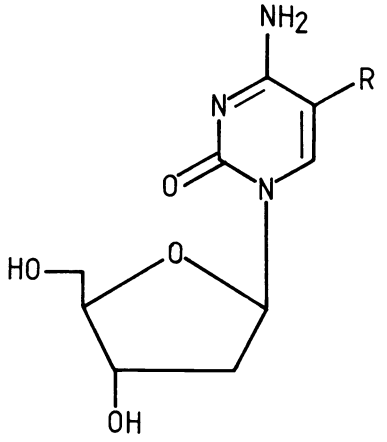
The (E)-5-(2-X-vinyl)-dUrd analogues were even less inhibitory for VV than for HSV_2. For (E)-5-(3,3,3-trifluoro-1-propenyl)-dUrd the difference in the ID_{50} for VV and ID_{50} for HSV_1 amounted to 5000-fold (Table 1). The ratio of ID_{50} for VV to ID_{50} for HSV_1 can be considered as another reliable parameter for the selectivity of the compounds as anti-HSV_1 agents. Of all 5-substituted dUrd analogues tested, (E)-5-(2-bromovinyl)-dUrd emerged as the most potent inhibitor of HSV_1 replication, whereas 5-vinyl-dUrd proved the most effective against HSV_2. The greatest selectivity was displayed by (E)-5-(2-chlorovinyl)-dUrd.

5-SUBSTITUTED 2'-DEOXYCYTIDINES

The 5-substituted dCyd derivatives (Fig. 2) inhibited HSV_1 replication at similar or slightly higher concentrations than their dUrd counterparts (Table 2). This appeared also to be the case for HSV_2 replication. Like the (E)-5-(2-X-vinyl)-dUrd analogues, (E)-5-(2-bromovinyl)-dCyd and (E)-5-(2-iodovinyl)-dCyd discriminated between HSV_1 and HSV_2, inhibiting HSV_2 at a concentration that was about 100-fold higher than the concentration required to inhibit HSV_1 replication (Ref. 16). The transition from the dUrd to the dCyd series was accompanied by a significant decrease in anti-VV activity. This decrease in anti-VV activity was particularly striking for 5-nitro-dCyd (Ref. 8) and 5-vinyl-dCyd (Ref. 17), which were 250- to 350-fold less active against vaccinia virus than the corresponding dUrd derivatives.

All 5-substituted dCyd that were examined proved to be selective inhibitors of HSV_1 replication. Those compounds that were highly selective antiherpes agents in their dUrd form, i.e. (E)-5-(2-bromovinyl)-dUrd and (E)-5-(2-iodovinyl)-dUrd, retained their selectivity when converted to the dCyd form; and those compounds that were not selective or "negatively" selec-

tive antiherpes agents in their dUrd form, i.e. 5-nitro-dUrd and 5-ethynyl-dUrd, acquired a significant increase in selectivity upon conversion to the dCyd form. Of all dCyd analogues tested, (E)-5-(2-bromovinyl)-dCyd was the most potent inhibitor of HSV_1 replication. It was also the most selective inhibitor. Akin to 5-vinyl-dUrd among the dUrd analogues, 5-vinyl-dCyd ranked as the most potent inhibitor of HSV_2 replication among the dCyd analogues.



R = I : 5-iodo-dCyd
NO_2 : 5-nitro-dCyd
C≡CH : 5-ethynyl-dCyd
$CH_2CH_2CH_3$: 5-propyl-dCyd
$CH=CH_2$: 5-vinyl-dCyd
CH=CHBr(E) : (E)-5-(2-bromovinyl)-dCyd
CH=CHI(E) : (E)-5-(2-iodovinyl)-dCyd

Fig. 2. 5-Substituted 1-(2-deoxy-β-D-ribofuranosyl)-cytosine (2'-deoxycytidine, dCyd) derivatives.

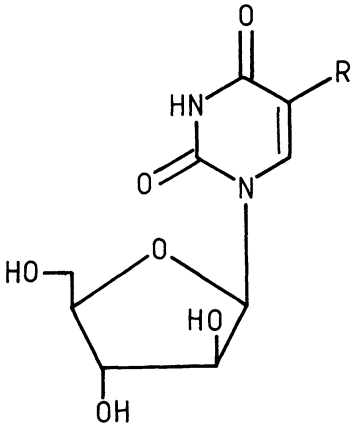
TABLE 2. Antiviral activity of 5-substituted 2'-deoxycytidines

Compound	ID_{50}[a] (μg/ml)			Selectivity index[b]
	HSV_1	HSV_2	VV	
5-Iodo-dCyd	0.09	0.37	4	100
5-Nitro-dCyd	0.67	2	70	15
5-Ethynyl-dCyd	0.3	1.7	5	10
5-Propyl-dCyd	7	30	> 400	≥ 29
5-Vinyl-dCyd	0.2	0.2	100	1000
(E)-5-(2-Bromovinyl)-dCyd (BVDC)	0.067	10	200	1492
(E)-5-(2-Iodovinyl)-dCyd	0.077	8	150	650

[a]Dose required to inhibit virus-induced cytopathogenicity in PRK cells by 50 %.
[b]Average values for 3 HSV_1 strains and 3 HSV_2 strains.
[b]Based on the ratio of ID_{50} for host cell metabolism (dose required to inhibit dUrd incorporation into DNA of uninfected PRK cells by 50 %) to ID_{50} for HSV_1.
Data taken from De Clercq *et al.* (16 & 17).

5-SUBSTITUTED URACIL ARABINOSIDES

For a number of 5-substituted dUrd analogues the corresponding 5-substituted araU analogues have been prepared (Fig. 3). As a rule, the 5-substituted araU analogues were less active against HSV_1 than their dUrd counterparts (Table 3); thus, (E)-5-(2-bromovinyl)-araU < (E)-(2-bromovinyl)-dUrd (Ref. 18), 5-nitro-araU < 5-nitro-dUrd (Ref. 19), 5-ethyl-araU < 5-ethyl-dUrd (Ref. 20), 5-vinyl-araU < 5-vinyl-dUrd (Ref. 21 & 22), 5-propyl-araU < 5-propyl-dUrd (Ref. 23), and (E)-5-(1-propenyl)-araU < (E)-5-(1-propenyl)-dUrd (Ref. 24). The only exception to this rule is araT (5-methyl-araU) which is a potent inhibitor of HSV replication whereas its dUrd counterpart, dThd, is not.



R = H : araU
NO_2 : 5-nitro-araU
CH_3 : 5-methyl-araU (araT)
CH_2CH_3 : 5-ethyl-araU
$CH_2CH_2CH_3$: 5-propyl-araU
$CH=CH_2$: 5-vinyl-araU
CH=CHCl(E) : (E)-5-(2-chlorovinyl)-araU
CH=CHBr(E) : (E)-5-(2-bromovinyl)-araU
$CH=CHCH_3$(E) : (E)-5-(1-propenyl)-araU

Fig. 3. 5-Substituted 1-(β-D-arabinofuranosyl)-uracil (uracil arabinoside, araU) derivatives.

TABLE 3. Antiviral activity of 5-substituted uracil arabinosides

Compound	ID_{50}[a] (μg/ml)			Selectivity index[b]
	HSV_1	HSV_2	VV	
AraU	43	46	> 200	> 10
5-Nitro-araU	> 200	...	> 200	...
5-Methyl-araU (araT)	0.25	0.5	5	1200
5-Ethyl-araU	2	15	> 200	> 100
5-Propyl-araU	> 400	> 400	> 400	...
5-Vinyl-araU	(0.3)	(1)	...	...
(E)-5-(2-Chlorovinyl)-araU	(0.1)	(>1000)	...	...
(E)-5-(2-Bromovinyl)-araU (BVaraU)	0.1	35	> 200	1000
(E)-5-(1-Propenyl)-araU	(10)	(320)	...	...

[a]Dose required to inhibit virus-induced cytopathogenicity in PRK cells by 50 %.
[b]Average values for a representative number of HSV_1 and HSV_2 strains. Based on the ratio of ID_{50} for host cell metabolism (dose required to inhibit dUrd incorporation into DNA of uninfected PRK cells by 50 %) to ID_{50} for HSV_1. Data taken from De Clercq et al. (4 & 18), Torrence et al. (19) and Kulikowski et al. (20), except for 5-vinyl-araU, (E)-5-(2-chlorovinyl)-araU and (E)-5-(1-propenyl)-araU. The latter data are taken from Machida et al. (21 & 22) and were obtained in HEF (instead of PRK) cells.

AraT was the only araU analogue which inhibited the replication of VV. Also, araT and 5-vinyl-araU were the only araU analogues showing an appreciable anti-HSV_2 activity. However, 5-vinyl-araU proved somewhat less inhibitory to HSV_2 than 5-vinyl-dUrd or 5-vinyl-dCyd. The most potent inhibitors of HSV_1 replication were, again, the (E)-5-(2-halogenovinyl)derivatives. (E)-5-(2-Bromovinyl)-araU inhibited the replication of HSV_1 in PRK cells at a concentration of 0.1 μg/ml, that is about 10-fold higher than the concentration required for (E)-5-(2-bromovinyl)-dUrd to inhibit HSV_1 replication in PRK cells. Like (E)-5-(2-bromovinyl)-dUrd, (E)-5-(2-bromovinyl)-araU showed a marked selectivity in its anti-HSV_1 activity and did not inhibit HSV_2 replication unless its concentration was raised to more than 100 times the HSV_1-inhibiting concentration.

ACTION SPECTRUM OF (E)-5-(2-BROMOVINYL)-dUrd

The preceding studies which were all performed in primary rabbit kidney cell cultures revealed that the (E)-5-(2-halogenovinyl)derivatives exceeded all other nucleoside analogues in anti-HSV_1 potency and selectivity. From these (E)-5-(2-halogenovinyl)derivatives BVDU could be singled out as the most potent and selective anti-HSV_1 agent. As shown in Table 4, its antiviral activity was not limited to rabbit cells. Its action spectrum extended to various other cell lines of either human, simian, feline or murine origin (Ref. 25). According to the cell line chosen the ID_{50} of BVDU for HSV_1 varied from 0.004 µg/ml (murine fibroblast cells) to 0.2 µg/ml (feline lung cells). A much greater variation of potency was observed for BVaraU which inhibited HSV_1 replication in human cells at a concentration of 0.01 µg/ml, while it failed to do so in simian cells at concentrations up to 200 µg/ml. Irrespective of the cell line chosen, BVDU was 10- to 20-fold more active against HSV_1 than the standard antiherpes drug IDU. BVDU was also more active than the other selective antiherpes drugs, ACV and FIAC, in human, simian and murine but not feline cells (Ref. 25).

TABLE 4. Antiviral activity of (E)-5-(2-bromovinyl)-dUrd (BVDU) and related compounds in different cell lines

Compound	ID_{50}[a] (µg/ml)				
	Origin of cell line				
	Mouse (MO)	Cat (FL)	Rabbit (PRK)	Monkey (Vero)	Human (HEF)
(E)-5-(2-Bromovinyl)-dUrd (BVDU)	0.004	0.2	0.007	0.1	0.01
(E)-5-(2-Bromovinyl)-araU (BVaraU)	0.15	0.04	0.1	200	0.01
5-Iodo-dUrd (IDU)	0.07	2	0.15	0.7	0.1
Acyclovir (ACV)	0.01	0.02	0.06	0.9	0.2
Fluoroiodoaracytosine (FIAC)	0.04	0.004	0.02	0.2	0.02

[a]Dose required to inhibit cytopathogenicity of HSV_1 (strain KOS) by 50 %. Data taken from De Clercq (25).

BVDU and a selected number of 5-substituted dUrd, dCyd and araU analogues were evaluated for their inhibitory effects on VZV replication in human embryo fibroblasts. From these studies, again, the (E)-5-(2-halogenovinyl)derivatives emerged as the most potent and selective antiviral agents (Ref. 26 & 27). Three compounds, (E)-5-(2-bromovinyl)-dUrd, (E)-5-(2-iodovinyl)-dUrd and (E)-5-(2-bromovinyl)-araU inhibited VZV replication at an ID_{50} of 1-2 ng/ml, that is even lower than the ID_{50} for HSV_1 replication. Moreover, these compounds did not affect normal cell metabolism (as monitored by incorporation of [1',2'-^{3}H]dThd into DNA) unless they were added at a concentration of 50-100 µg/ml, thus 50,000 times the minimal antiviral concentration (Table 5). As compared to BVDU, BVDC was 10 times less potent and also ten times less selective as an anti-VZV agent. In turn, araT was 10 times less potent and selective than BVDC, and another ten-fold drop in potency and selectivity was noted for ACV. Thus, ACV appeared to be 1000 times less potent and selective in its anti-VZV activity than BVDU. This is the more remarkable as ACV has been launched as a promising chemotherapeutic agent for the treatment of VZV infections in humans (Ref. 28 & 29). From our own studies (Ref. 26 & 27) and those of Machida *et al.* (30), the (E)-5-(2-halogenovinyl)derivatives BVDU and BVaraU would appear much more promising for clinical application than ACV.

The activity spectrum of BVDU is not restricted to HSV_1 and VZV but extends to several other herpesviruses such as pseudorabies virus (PRV), bovid herpesvirus type 1 (BHV_1), simian varicella virus (SVV), herpesvirus saimiri (HVS) and nuclear polyhedrosis virus (NPV) (Table 6) (Ref. 3, 4, 27 & 31-35), HVS being a simian lymphotropic virus and NPV, an invertebrate pathogenic virus. BVDU inhibited the replication of PRV, BHV_1 and SVV at a concentration of 0.01 µg/ml, that is the same concentration as that required for inhibition of HSV_1 replication. Although ACV proved effective against HSV_1 and HSV_2 at a concentration of 0.04 µg/ml, it had no effect on the replication of PRV, BHV_1, HVS and NPV (Ref. 31, 32, 34 & 35). This lack of activity may be related to the fact that the dThd kinase encoded by PRV, BHV_1, HVS and NPV is devoid of dCyd kinase activity and that ACV needs this dCyd kinase activity to express its selective antiviral action. The dThd kinase encoded by HSV_1 and HSV_2 has an associated dCyd kinase activity and this would explain why ACV is specifically active against these viruses. Since no dCyd kinase activity is induced in PRV-, BHV_1-, HVS- and NPV-infected cells, ACV cannot be phosphorylated in these cells, and, consequently, fail to interact with the virus replicative cycle. BVDU would still be effective under these conditions, since its phosphorylation depends on the virus-induced dThd kinase rather than dCyd kinase.

TABLE 5. Potency and selectivity of (E)-5-(2-bromovinyl)-dUrd (BVDU) and related compounds as inhibitors of VZV replication

Compound	ID_{50}[a] (μg/ml)		Selectivity index[b]
	VZV	Host cell metabolism	
5-Iodo-dUrd (IDU)	1.38	0.8	0.58
5-Ethyl-dUrd	1.47	24	16.3
(E)-5-(2-Bromovinyl)-dUrd (BVDU)	0.0024	100	41666
(E)-5-(2-Iodovinyl)-dUrd	0.0015	100	66667
5-Iodo-dCyd	1.33	2.7	2.0
(E)-5-(2-Bromovinyl)-dCyd (BVDC)	0.023	100	4348
5-Methyl-araU (araT)	0.23	61	265
(E)-5-(2-Bromovinyl)-araU (BVaraU)	0.0013	66	50769
Acyclovir (ACV)	4.64	180	39

[a]Dose required to inhibit VZV focus formation in HEF cells (average value for 10 VZV strains), or dThd incorporation into DNA of uninfected HEF cells by 50 %.
[b]Based on the ratio of ID_{50} for host cell metabolism (dThd incorporation into DNA) to ID_{50} for VZV.
Data taken from Shigeta et al. (27).

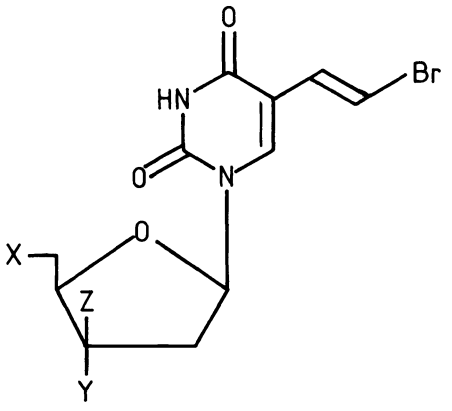
TABLE 6. Spectrum of antiviral activity of (E)-5-(2-bromovinyl)-dUrd (BVDU)

Virus	Cell line	ID_{50}[a] (μg/ml)	References
HSV_1 (herpes simplex virus type 1)	PRK, HEF	0.007-0.01	De Clercq et al. (3)
HSV_2 (herpes simplex virus type 2)	PRK	1	De Clercq et al. (4)
VZV (varicella zoster virus)	HEF	0.0024	Shigeta et al. (27)
PRV (pseudorabies virus)	BHK, Vero	0.006-0.06	Reefschläger et al. (31)
BHV_1 (bovid herpesvirus type 1)	MDBK	0.01	Weinmaster et al. (32)
SVV (simian varicella virus)	Vero	0.01	Soike (33)
HVS (herpesvirus saimiri)	OMK	$\ll$ 10	Honess et al. (34)
NPV (nuclear polyhedrosis virus)	SF	0.5	Wang et al. (35)

[a]Dose required to inhibit viral cytopathogenicity, focus or plaque formation by 50 %.

SUGAR-MODIFIED DERIVATIVES OF (E)-5-(2-BROMOVINYL)-dUrd

With (E)-5-(2-bromovinyl)-dUrd (BVDU) as starting material several sugar-modified derivatives (Fig. 4) were prepared in attempts to develop, if at all possible, a compound with superior anti-HSV_2 activity. Of this series of BVDU derivatives, only three compounds, 3'-azido-BVDU, 3'-amino-BVDU and 5'-iodo-BVDU, exhibited an appreciable antiherpes activity (Table 7) (Ref. 18 & 36). These compounds inhibited HSV_1 replication at a concentration of 1, 0.1 and 1 μg/ml, respectively. None of the new sugar-modified derivatives of BVDU was more active against HSV_2 than the parent compound. For 3'-amino-BVDU the ratio of ID_{50} for HSV_2 to ID_{50} for HSV_1 amounted to 250, which implies that this compound, like BVDU and the other (E)-5-(2-halogenovinyl)substituted dUrd, dCyd and araU analogues could be proposed as useful markers to discriminate between HSV_1 and HSV_2. 3'-Amino-BVDU could be regarded as a truly selective inhibitor of HSV_1. This was further attested by its lack of anti-VV activity. Even at a concentration of 200 μg/ml, that is 2000 times higher than the ID_{50} for HSV_1, 3'-amino-BVDU failed to inhibit the replication of vaccinia virus. So did most other sugar-modified derivatives of BVDU.



X	Y	Z	Compound
OH	OH	H	BVDU
OH	Cl	H	3'-chloro-BVDU
OH	N_3	H	3'-azido-BVDU
OH	NH_2	H	3'-amino-BVDU
Cl	OH	H	5'-chloro-BVDU
Br	OH	H	5'-bromo-BVDU
I	OH	H	5'-iodo-BVDU
N_3	OH	H	5'-azido-BVDU
NH_2	OH	H	5'-amino-BVDU
OH	H	OH	BVxyloDU
OH	H	N_3	3'-azido-BVxyloDU
OH	H	NH_2	3'-amino-BVxyloDU

Fig. 4. Sugar-modified derivatives of (E)-5-(2-bromovinyl)-dUrd (BVDU).

TABLE 7. Antiviral activity of sugar-modified derivatives of (E)-5-(2-bromovinyl)-dUrd (BVDU)

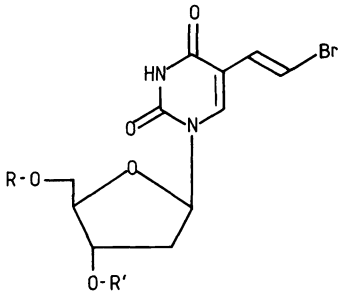
Compound	ID_{50}[a] (μg/ml)		
	HSV_1	HSV_2	VV
BVDU	0.008	1	7
3'-Chloro-BVDU	> 200	> 200	> 200
3'-Azido-BVDU	1	50	200
3'-Amino-BVDU	0.1	25	> 200
5'-Chloro-BVDU	≥ 100	≥ 200	> 200
5'-Bromo-BVDU	100	100	...
5'-Iodo-BVDU	1	10	70
5'-Azido-BVDU	> 200	≥ 200	> 200
5'-Amino-BVDU	40	> 200	> 200
BVxyloDU	> 200	> 200	> 200
3'-Azido-BVxyloDU	200	150	> 200
3'-Amino-BVxyloDU	20	> 200	> 200

[a]Dose required to inhibit virus-induced cytopathogenicity in PRK-cells by 50 %. Average values for 3 HSV_1 strains and 3 HSV_2 strains.
Data taken from Busson et al. (36) and De Clercq et al. (18).

3'-O- AND 5'-O-ESTER DERIVATIVES OF (E)-5-(2-BROMOVINYL)-dUrd

Several 3'-O-, 5'-O- and 3',5'-di-O-acyl esters of (E)-5-(2-bromovinyl)-dUrd (BVDU) (Fig. 5) have been prepared, principally aimed at changing the pharmacological properties (i.e. solubility in aqueous or lipid medium) of BVDU. With the exception of the highly lipophilic esters, 5'-O-palmitoyl-BVDU, 5'-O-adamantanocarbonyl-BVDU and 3',5'-di-O-pivaloyl-BVDU, all BVDU esters inhibited the replication of HSV_1 at an ID_{50} which was only slightly higher than the ID_{50} of the parent compound (Table 8). This suggests that under the conditions used the BVDU esters were readily hydrolyzed to release the free nucleoside. The ID_{50} of the BVDU esters for HSV_2 was 100- to 500-fold higher than the ID_{50} for HSV_1, which, again, indicated that their antiviral activity resided in the release of free BVDU and that the BVDU esters, like BVDU itself, could serve as useful markers to differentiate HSV_1 from HSV_2 strains. For some BVDU esters the anti-VV activity was comparable to that of BVDU; for others; espe-

cially the lipophilic ones, no activity was noted against VV. It would now seem mandatory to examine whether any of the 3'-O-, 5'-O- or 3',5'-di-O-acyl esters of BVDU offers a greater therapeutic benefit than the parent compound in animal model infections.



R	R'	Compound
H	H	BVDU
$COCH_2NH_2.HCl$	H	5'-O-aminoacetyl-BVDU.HCl
$COCH_2N_3$	H	5'-O-azidoacetyl-BVDU
$COC(CH_3)_3$	H	5'-O-pivaloyl-BVDU
$COCH_2OC_6H_5$	H	5'-O-phenoxyacetyl-BVDU
H	$CO(CH_2)_3CH_3$	3'-O-valeryl-BVDU
$COCH_3$	$COCH_3$	3',5'-di-O-acetyl-BVDU
$COC(CH_3)_3$	$COC(CH_3)_3$	3',5'-di-O-pivaloyl-BVDU
$COC(CH_3)_3$	$COCH_2NH_2.HCl$	3'-O-aminoacetyl-5'-O-pivaloyl-BVDU.HCl
$COCH_2NH_2.HCl$	$COC(CH_3)_3$	3'-O-pivaloyl-5'-O-aminoacetyl-BVDU.HCl
$CO(CH_2)_{14}CH_3$	H	5'-O-palmitoyl-BVDU
CO	H	5'-O-adamantanocarbonyl-BVDU

Fig. 5. 3'-O- and 5'-O-Ester derivatives of (E)-5-(2-bromovinyl)-dUrd (BVDU).

TABLE 8. Antiviral activity of 3'-O- and 5'-O-ester derivatives of (E)-5-(2-bromovinyl)-dUrd (BVDU)

Compound	ID_{50}[a] (μg/ml)		
	HSV_1	HSV_2	VV
BVDU	0.008	1	7
5'-O-Aminoacetyl-BVDU.HCl	0.01	2	20
5'-O-Azidoacetyl-BVDU	0.02	7	4
5'-O-Pivaloyl-BVDU	0.02	3	8
5'-O-Phenoxyacetyl-BVDU	0.02	8	8
3'-O-Valeryl-BVDU	0.03	2.5	> 40
3',5'-di-O-Acetyl-BVDU	0.05	3.7	> 10
3',5'-di-O-Pivaloyl-BVDU	0.23	26	> 100
3'-O-Aminoacetyl-5'-O-pivaloyl-BVDU.HCl	0.007	4	70
3'-O-Pivaloyl-5'-O-aminoacetyl-BVDU.HCl	0.03	3.2	20
5'-O-Palmitoyl-BVDU	2.7	> 40	> 40
5'-O-Adamantanocarbonyl-BVDU	2	> 40	> 40

[a]Dose required to inhibit virus-induced cytopathogenicity in PRK cells by 50 %. Average values for 3 HSV_1 strains and 3 HSV_2 strains.
Data taken from De Clercq et al. (37).

MECHANISM OF ANTIVIRAL ACTION OF (E)-5-(2-BROMOVINYL)-dUrd AND OTHER (E)-5-(2-BROMOVINYL) DERIVATIVES

The mode of action of (E)-5-(2-bromovinyl)-dUrd has been elucidated to a considerable extent. It is essentially based on three principles : (i) a specific phosphorylation by the virus-induced dThd kinase, (ii) a greater inhibition of viral DNA polymerase than cellular DNA polymerase by BVDU 5'-triphosphate (BVDUTP), and (iii) the incorporation of the latter into viral DNA (Fig. 6).

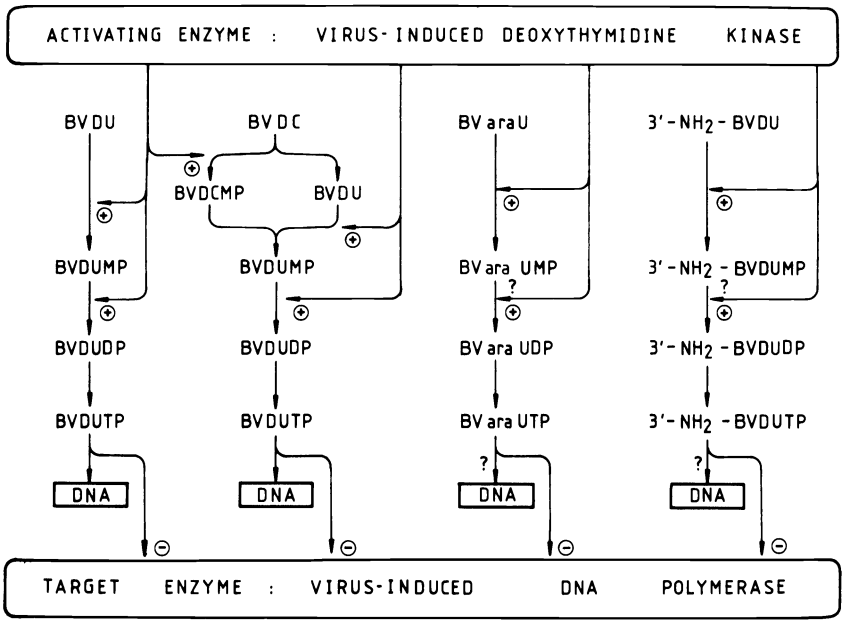


Fig. 6. Mechanism of antiviral action of BVDU ((E)-5-(2-bromovinyl)-dUrd) and its congeners BVDC ((E)-5-(2-bromovinyl)-dCyd), BVaraU ((E)-5-(2-bromovinyl)-araU) and 3'-NH_2-BVDU ((E)-5-(2-bromovinyl)-3'-amino-2',3'-dideoxyuridine).

BVDU has a much higher affinity for the HSV_1-, HSV_2- and VZV-induced dThd kinase (K_i = 0.24, 4.24 and 0.07 µM, respectively) than for the cellular dThd kinase (K_i > 150 µM) (Ref. 38). This explains why the (E)-5-(2-halogenovinyl)derivative is converted to its 5'-monophosphate in virus-infected cells, whereas such phosphorylation does not occur in uninfected cells (Ref. 39). The HSV_1-induced dThd kinase, but not the HSV_2-induced dThd kinase, would be endowed with an associated dTMP kinase activity (Ref. 40), capable of converting BVDU 5'-monophosphate (BVDUMP) to BVDU 5'-diphosphate (BVDUDP). The fact that the HSV_2-encoded dThd kinase lacks this dTMP kinase activity may obviously bear on the differential susceptibilities of HSV_1 and HSV_2 to the antiviral effects of BVDU.

The target for the antiviral action of BVDU may be viral DNA synthesis or viral DNA itself. Indeed, the 5'-triphosphate of BVDU has been shown to inhibit HSV_1 DNA polymerase to a significantly greater extent than the cellular DNA polymerases α, β and γ (Ref. 41). Moreover, BVDUTP can substitute for dTTP as an alternate substrate for both viral and cellular DNA polymerases (Ref. 42 & 43), and thus be incorporated into DNA. BVDU is not incorporated into DNA of uninfected mouse (BALB/3T3) or monkey (Vero) cells (Ref. 42 & 44), most likely because it is not phosphorylated in these cells. However, BVDU is incorporated into DNA of HSV_1-infected cells. If added at a supra-optimal concentration (10 µg/ml), it is incorporated into both viral and cellular DNA of HSV_1-infected Vero cells (Ref. 42). If added at optimal concentrations (0.03-0.1 µg/ml), it is incorporated into viral DNA only (Ref. 45). The extent of incorporation correlates closely with the reduction in virus yield, and since the BVDU-substituted HSV_1 DNA is liable to single-strand breakage (Ref. 45), the incorporation of BVDU into viral DNA may well be the decisive factor in the antiviral action of the compound.

Although the mechanism of action of the other (E)-5-(2-halogenovinyl)derivatives, i.e. (E)-5-(2-bromovinyl)-dCyd (BVDC), (E)-5-(2-bromovinyl)-araU (BVaraU) and 3'-amino-BVDU, has not been explored in detail, it is conceivable that they act in a similar manner as BVDU (Fig. 6). Therefore BVDC should first be deaminated at either the nucleoside or nucleotide level. An important difference in the mode of action of BVDU and BVaraU concerns their incorporation into DNA : while BVDUTP is recognized by DNA polymerase as an alternate substrate and is incorporated into DNA probably within the interior of the DNA chain, BVaraUTP does not serve as an efficient substrate for DNA polymerase, although it could be incorporated at the 3'-end of the DNA chain, thereby acting as a chain terminator (Ref. 46 & 47). Whether 3'-NH_2-BVDUTP is incorporated into DNA has not been determined yet. Unlike BVDUTP, BVaraUTP and 3'-NH_2-BVDUTP do not inhibit HSV_1 DNA polymerase to a greater extent than cellular DNA polymerase α (Ref. 47 & 48). One may postulate, therefore, that the selectivity of BVaraU and 3'-NH_2-BVDU as antiherpes agents depends primarily on a specific phosphorylation

by the viral dThd kinase. This would be consistent with the high affinity of BVaraU for HSV_1 dThd kinase (K_i = 0.94 μM, as compared to > 100 μM for cytosol dThd kinase) (Ref. 49). Whether the HSV_1 dThd carries on the phosphorylation of BVaraU and 3'-NH_2-BVDU to the 5'-diphosphate stage, as shown for BVDU, remains to be established.

EFFICACY OF (E)-5-(2-BROMOVINYL)-dUrd IN ANIMAL MODEL INFECTIONS

From the (E)-5-(2-halogenovinyl)derivatives, (E)-5-(2-bromovinyl)-dUrd (BVDU) was chosen for further evaluation in experimental animal models, i.e., cutaneous and intracerebral HSV_1 infection in mice, HSV_1 keratitis and uveitis (iritis) in rabbits, SVV infection in monkeys and PRV infection in pigs (Table 9) (Ref. 3 & 50-62). Both topical and systemic administration of BVDU suppressed the development of cutaneous HSV_1 lesions, and mortality associated therewith, in athymic-nude mice (Ref. 3, 50 & 52). Similarly, BVDU protected hairless mice against a lethal HSV_1 infection, but no protection was achieved with BVDU in HSV_2-infected mice (Ref. 51). BVDU prevented the establishment of latent HSV infections (Ref. 53 & 54). However, latent HSV_1 infections once established were not amenable to BVDU therapy. Nor were they amenable to ACV therapy (Ref. 53 & 54). In mice inoculated intracerebrally with HSV_1, BVDU effected a significant reduction in the mortality rate if treatment was initiated shortly after virus infection (Ref. 55). In guinea pigs BVDU significantly reduced the development of HSV_1 skin lesions when applied topically at 3 % in polyethylene glycol (Ref. 56).

TABLE 9. Animal model infections in which (E)-5-(2-bromovinyl)-dUrd (BVDU) has shown efficacy

Virus	Disease	Animals	Administration[a]	Dosage	References
HSV_1	Cutaneous herpes	Mice	ointment	1 %	De Clercq et al. (3) Descamps et al. (50)
				5 %	De Clercq & Zhang (51)
			p.o., i.p.	60-300 mg/kg	De Clercq et al. (3 & 52) De Clercq & Zhang (51)
HSV_1	Mucocutaneous herpes	Mice	ointment	3-5 %	Park et al. (53)
			p.o., i.p., s.c.	50-100 mg/kg	Field & De Clercq (54) Park et al. (53)
HSV_1	Herpetic encephalitis	Mice	p.o., i.p., s.c.	40-400 mg/kg	De Clercq et al. (55)
HSV_1	Cutaneous herpes	Guinea pigs	ointment	3 %	Sim et al. (56)
HSV_1	Herpetic keratitis	Rabbits	ointment	0.1-2.5 %	Maudgal et al. (57 & 58)
			eye drops	0.1-0.5 %	Maudgal et al. (58 & 59)
	Herpetic iritis	Rabbits	eye drops	0.5 %	Maudgal et al. (60)
			p.o.	10 mg/kg	Maudgal et al. (60)
SVV	Varicella	Monkeys	p.o., i.m., i.v.	5-15 mg/kg	Soike et al. (61)
PRV	Pseudorabies	Pigs	p.o.	25 mg/kg	Biront & De Clercq (62)

[a] p.o., perorally; i.p., intraperitoneally, s.c., subcutaneously; i.m., intramuscularly; i.v., intravenously.

Under both eye drop or ointment formulations, BVDU proved superior to the standard antiherpes drug, IDU, in promoting the healing of epithelial HSV_1 keratitis in rabbits (Ref. 57 & 58). BVDU eye drops were also effective in the topical treatment of stromal HSV_1 keratitis, and in this regard BVDU proved superior to another classical antiherpes drug, TFT (if treatment was started one day after infection) (Ref. 59). Favorable results have also been obtained with both topical and systemic administration of BVDU in the treatment of herpetic uveitis (iritis), produced by injection of HSV_1 into the anterior chamber of rabbit eyes (Ref. 60).

In African green monkeys infected with SVV, BVDU eliminated all manifestations of the disease, including viremia, rash and anorexia, when given at a dosage as low as 15 mg/kg/day; but, even if the dosage was lowered to 1 mg/kg/day, BVDU conferred a slight inhibitory effect on the disease (Ref. 61). Preliminary observations indicate that BVDU would also be effective in mitigating the symptoms, i.e., fever, weight loss and death rate, associated with a pseudorabies virus infection of pigs (Ref. 62).

Whenever different routes of BVDU administration were explored (Ref. 55 & 61), the peroral route proved at least as effective as either intraperitoneal, subcutaneous, intramuscular or intravenous injection. Oral administration may well be the route of choice for the systemic treatment of herpesvirus infections by BVDU.

EFFICACY OF (E)-5-(2-BROMOVINYL)-dUrd IN HUMANS

Prompted by the efficacy of (E)-5-(2-bromovinyl)-dUrd (BVDU) in animal model infections and its apparent freedom of toxicity at therapeutically effective doses, clinical trials have been initiated to assess the usefulness of BVDU in the topical and systemic (oral) treatment of HSV_1 and VZV infections in humans (Table 10). The drug proved highly efficacious, as 0.1 % eye drops, in the topical treatment of patients with different forms of herpetic keratitis, i.e. dendritic corneal ulcers, geographic corneal ulcers and stromal disease (Ref. 63 & 64). Most of these patients had first been treated with topical IDU or ara-A (vidarabine, 9-β-D-arabinofuranosyladenine), albeit unsuccessfully, before BVDU treatment was started. They all responded rapidly to BVDU therapy. No toxic side effects, whether local or systemic, were noted in any of the patients treated with BVDU.

TABLE 10. Clinical conditions in which (E)-5-(2-bromovinyl)-dUrd (BVDU) has shown efficacy

Virus	Disease	Administration	Dosage	References
HSV_1	Herpetic keratitis	eye drops	0.1 %	Maudgal et al. (63 & 64)
HSV_1	Mucocutaneous herpes simplex in immunosuppressed patients	perorally	7.5 mg/kg	Tricot et al. (65)
VZV	Ophthalmic zoster	eye drops	0.1 %	Maudgal et al. (64 & 66)
		perorally	7.5 mg/kg	Maudgal et al. (64 & 66)
VZV	Varicella-zoster in leukemic children	perorally	15 mg/kg	Benoit et al. (67)
VZV	Severe herpes zoster in cancer patients	perorally	7.5 mg/kg	De Clercq et al. (68) Wildiers et al. (69)

BVDU was also administered perorally at a dosage of 7.5-15 mg/kg/day for 5 days to cancer patients who had developed a severe mucocutaneous herpes simplex or herpes zoster infection as the consequence of an intensive anticancer chemotherapy or radiotherapy (Ref. 65, 68 & 69). In these patients BVDU caused a rapid healing of the herpes lesions : pain and fever (if present) subsided, no new lesions formed and existing lesions regressed within the first few days after BVDU treatment was started. Similar promising results have been obtained with oral BVDU (combined with BVDU eye drops) in the treatment of patients suffering from ophthalmic zoster (Ref. 64 & 66). Equally successful were results of oral BVDU treatment in leukemic children with severe varicella or zoster (Ref. 67). All children recovered promptly from their varicella-zoster episode. Neither adults nor children showed any sign of drug toxicity for liver, kidney, bone marrow or any other organ.

It should be emphasized that the clinical studies that have so far been conducted with BVDU (Ref. 63-69) were all uncontrolled. Obviously, these studies should be extended to double-blind placebo-controlled trials before the therapeutic usefulness of BVDU could be fully substantiated.

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