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EFFICIENCY AND SELECTIVITY OF (*E*)-5-(2-BROMOVINYL)-2′-DEOXYURIDINE AND SOME OTHER 5-SUBSTITUTED 2′-DEOXYPYRIMIDINE NUCLEOSIDES AS ANTI-HERPES AGENTS

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A number of novel 5-substituted 2′-deoxypyrimidine nucleosides exhibited antiviral activity against herpes simplex virus type 1 strain V3 (HSV-1-V3) when assayed under one-step conditions in primary human lung fibroblast (PHLF) cell cultures, and compared with the reference compounds cytosine arabinoside (ara-C), 5-iodo-2′-deoxyuridine (IUdR), and 5-iodo-5′-amino-2′,5′-dideoxyuridine (AIU). The most effective of these were (in order of decreasing activity): (*E*)-5-(2-bromovinyl)-UdR (BrVUdR) > ara-C > IUdR > 5-azidomethyl-UdR (AMeUdR) > 5-formyl-UdR (fUdR) > 5-hydroxymethyl-UdR (HMeUdR) > AIU > 5-mercaptomethyl-UdR (MMeUdR) = 5-hydroxymethyl-2′-deoxycytidine (HMeCdR) > 5-benzyloxymethyl-UdR (BOMeUdR).

In a multistep virus replication experiment (plaque reduction assay on Vero cells) the order of decreasing activity was as follows: BrVUdR = ara-C > HMeUdR > fUdR = IUdR > HMeCdR > BOMeUdR > AMeUdR > AIU > MMeUdR.

BrVUdR effected a 50% reduction in plaque formation of different strains of HSV-1 at a concentration of 0.06–0.22 μM, of pseudorabies virus (PRV) at 0.02–0.23 μM, and of herpes simplex virus type 2 (HSV-2) at 8 μM, whereas the ID_{50} values for adenovirus type 2 and type 5 were 100 and 50–100 μM, respectively. The growth of synchronized baby hamster kidney cells in suspension cultures was inhibited by 50% at concentrations of 100, 70, 20, 4, 8, and 0.2 μM for BrVUdR, HMeCdR, IUdR, fUdR, BOMeUdR, and HMeUdR, respectively.

(*E*)-5-(2-bromovinyl)-2′-deoxyuridine 5-substituted-2′-deoxypyrimidine nucleosides herpes simplex virus strains structure–activity relationship pseudorabies virus

INTRODUCTION

Of the great number of antiherpetic 5-substituted pyrimidine nucleosides described during the last years (for review, see refs. 5 and 17), some show selectivity in that they are not cytotoxic at effective antiviral concentrations. This class of compounds is not only promising with regard to possible practical applications, but also suited to study some principles of antiviral selectivity as dependent on inhibitor structure.

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In this paper we report data on the antiviral properties and selectivity of some 5-substituted 2′-deoxypyrimidine nucleosides, including (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BrVUdR). For some of the compounds (Nos. 1, 2, 10, 11, 14 in Fig. 1) antiviral activities have been described previously, either by us [24, 34] or by others [7, 8, 12, 15, 20]. We have now compared their effects with those of a series of structurally related compounds. This study and previous data reported in the literature have enabled us to draw some conclusions as to the structure–activity relationship of 5-substituted pyrimidine nucleosides. A structure–activity function relationship study has already been reported by De Clercq [6]. The results also demonstrate that the outcome of antiviral studies to some extent depends on the assay system used (one-step or plaque reduction experiments).

Based on the data obtained in both systems BrVUdR emerged as the most promising antiherpes compound and has been evaluated in more detail. Using several herpesvirus strains, we have been able to confirm the extraordinarily high antiherpetic properties of BrVUdR found by De Clercq et al. [7, 8, 11], who used other cell lines and an other antiviral assay (inhibition of cytopathogenic effect). In addition, we describe the effects of BrVUdR on adenovirus types 2 and 5, its very strong inhibitory effect on pseudorabies virus and its low and reversible cytostatic effect for the host cells (BHK).

MATERIALS AND METHODS

Test compounds

All substances (see Fig. 1) were synthesized as described previously [2, 25] with the exception of 5-hydroxymethyl-CdR which was prepared by transformation of 5-hydroxymethyl-UdR with hexamethyldisilazane (D. Bärwolff, manuscript in preparation). BrVUdR was prepared from 5-ethyl-(3′,5′-di-*O*-acetyl)-2′-deoxyuridine which was converted with two equivalents of bromine [2] to 5-(1,2-dibromoethyl)-(3′,5′-di-*O*-acetyl)-2′-deoxyuridine. The following elimination by triethylamine yields (*E*)-5-(2-bromovinyl)-2′-deoxyuridine. The two standard anti-herpes compounds 5-iodo-2′-deoxyuridine (IUdR) and 1-β-D-arabinofuranosylcytosine (ara-C) were purchased from Serva (Heidelberg, F.R.G.).

5-Iodo-5′-amino-2′,5′-dideoxyuridine was a generous gift from W.H. Prusoff (Department of Pharmacology, Yale University School of Medicine, New Haven, CT). For virus inhibition tests and tissue culture studies 20 mM stocks and dilutions were prepared in phosphate-buffered solution (PBS) and stored at -20°C.

Cells and cell culture techniques

The following cell lines were used: Vero (African green monkey kidney), primary human lung fibroblasts prepared from a 3–4 month old embryo (PHLF), BHK 21/C13 baby hamster kidney, strain 21, clone 13), BHK 21/C13/2P (suspension line), and primary chicken embryo fibroblasts (CEF).

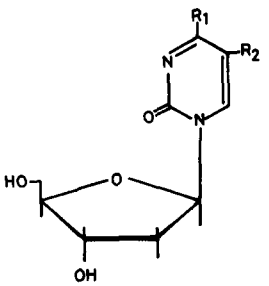

Fig. 1. 5-Substituted 2'-deoxypyrimidine nucleosides.

R_1 = OH; R_2 = CH_2-N_3	No. 1	5-azidomethyl-UdR
R_1 = OH; R_2 = CH_2-SH	2	5-mercaptomethyl-UdR
R_1 = OH; R_2 = $CH_2-OCH_2-C_6H_5$	3	5-benzyloxymethyl-UdR
R_1 = OH; R_2 = CH_2-CN	4	5-cyanomethyl-UdR
R_1 = OH; R_2 = $CH_2-NHCH_2-C_6H_5$	5	5-benzylaminomethyl-UdR
R_1 = OH; R_2 = CH_2-SCN	6	5-thiocyanomethyl-UdR
R_1 = OH; R_2 = $CH_2-SCH_2CH_3$	7	5-ethylmercaptomethyl-UdR
R_1 = OH; R_2 = $CH_2-OC(CH_3)_3$	8	5-tertiarybutoxymethyl-UdR
R_1 = OH; R_2 = CH_2-N (CH_2, CH_2)	9	5-ethyleneiminomethyl-UdR
R_1 = OH; R_2 = CH_2OH	10	5-hydroxymethyl-UdR
R_1 = OH; R_2 = CHO	11	5-formyl-UdR
R_1 = OH; R_2 = $CH=N-NHCSNH_2$	12	5-thiosemicarbazone of 5-formyl-UdR
R_1 = OH; R_2 = $CH=N-NH-C_6H_3(NO_2)_2$	13	5-dinitrophenylhydrazone of 5-formyl-UdR
R_1 = OH; R_2 = CH=CHBr	14	5-(2-bromovinyl)-UdR
R_1 = NH_2 R_2 = CH_2OH	15	5-hydroxymethyl-CdR

Vero and BHK 21/C13 cells were received from the American Type Culture Collection (Rockville, U.S.A.). BHK 21/C13/2P cells were kindly supplied by P.B. Capstick (The Animal Virus Research Institute, Pirbright, Surrey, U.K.), and PHLF cells were provided by H. Giese (Institut für Angewandte Virologie, Berlin, G.D.R.).

The various cell lines were cultured at 37°C in monolayers with Eagle's minimal essential medium (EMEM; Institut für Immunpräparate und Nährmedien, Berlin, G.D.R.) and Earle's salts supplemented with 10% fetal calf serum (Gibco Bio-Cult Laboratory, Glasgow, U.K.) (EMEM/10) and, additionally, 10% tryptose phosphate broth (Oxoid, London, U.K.) (EMEM/10/10) for baby hamster kidney cell lines. Neomycin (Spofa, Prague, Czechoslovakia) was used as an antibiotic. BHK 21/C13/2P cells were grown in suspension cultures as described previously [28]. In brief, the cells were cultivated in EMEM/10/10 and Earle's salts without calcium ions, supplemented with 0.1% methylcellulose (Methocel, 4000 CPS, Dow Chemical Co., Midland, MI) using a special 100 ml glass vessel with a magnetic stirrer.

Inhibition of cell proliferation

All assays were performed with synchronized cultures of BHK 21/C13/2P suspension cells growing with a doubling time of nearly 12 h. As was previously shown by pulse cytophotometry as well as estimations of cell growth and DNA synthesis rates (incorporation of tritiated thymidine) up to 90% of the cells in the stationary phase of growth are in the G_1 phase of the cell cycle (J. Reefschläger, doctor thesis, 1977, Humboldt-Universität zu Berlin, G.D.R.). Batch cultures of G_1 phase cells of 10 ml each (cell density of 1.5×10^5 cells/ml) were incubated with appropriate concentrations of the analogs for a period corresponding to three generations of an untreated control cell culture. In experiments aimed at reversing the inhibitory effect of a substance, cell suspensions were centrifuged at the end of the drug treatment and washed once with prewarmed medium. The cells were then resuspended to the same cell density as the control culture. Cell counts were determined in duplicate for both treated and untreated cultures with a particle counter (Granulometer TuR ZG2, VEB Transformatoren- und Röntgenwerk, Dresden, G.D.R.). The cytostatic effect of drugs (ID_{50}, 50% inhibitory dose) was expressed as the concentration of compound that reduced the number of cell generations in a treated culture by 50% as compared to the number of cell generations in an untreated control culture. The inhibition data were plotted as dose–effect curves (not shown) from which the ID_{50} were obtained.

Viruses

DNA viruses. Herpes simplex virus type 1 (HSV-1), strains F, Cl 101, V3 and Kupka; herpes simplex virus type 2 (HSV-2), strain US; adenovirus type 1 (Adeno-1) strain adenoid 71, adenovirus type 2 (Adeno-2) strain adenoid 6, and adenovirus type 5 (Adeno-5); vaccinia virus (VV) strain Berlin; pseudorabies virus strain BUK (PRV-BUK) [31]. *RNA viruses*. Vesicular stomatitis virus (VSV); Sindbis virus (SbV); fowl-plague virus (FPV).

HSV-1 strain F and Cl 101 (lyophilized) were kindly supplied by W.H. Prusoff (Department of Pharmacology, Yale University School of Medicine, New Haven, CT). The PRV-BUK strain was received from the Institute of Virology, Slovakian Academy of Sciences, Bratislava, Czechoslovakia.

Inhibition of plaque formation

Plaque assays were performed in 20 ml scintillation vials (diameter 2.5 cm; Firma Müller, Neuhaus/Rennsteig, G.D.R.) using confluent monolayer cultures. Cells were infected with 0.1 ml virus suspension yielding nearly 100 plaques per vial. After a 1 h virus adsorption period, doubling concentrations of the test compounds (0.05 ml) in a 1 ml methocel (0.5% w/v) overlay were added to each cell culture. Macroscopic plaques were enumerated after addition of a neutral red solution to the overlay. The resulting

plaque counts were expressed as percentages of the counts obtained for untreated control cell cultures and plotted in function of the logarithm of the concentration. ID_{50} values were obtained from the dose–response curves. Triplicate cultures were used for each concentration.

Inhibition of virus one-step growth

Confluent PHLF cultures grown in scintillation vials were used as host cells (5×10^5 cells/vial/ml, seeded 24 h before infection) and infected with 5 plaque-forming units (p.f.u.)/cell of HSV-1-V3. After a 1 h adsorption period, the cultures were incubated for 24 h in 1 ml of Eagle's MEM/10 containing appropriate concentrations of the compounds, frozen with liquid nitrogen and thawed before virus titration by the plaque assay technique on Vero cells. The virus yields of treated cultures were compared with those of untreated cultures. The logarithms of yield reduction indicated in Table 1 represent the mean value of two or more experiments with duplicate samples. The increase of the virus titer during the 24 h incubation period was 2.85 ± 0.49 log p.f.u./0.1 ml culture fluid.

RESULTS

Comparison of antiviral potency of 5-substituted 2'-deoxypyrimidine nucleosides

In an initial series of experiments the influence of fifteen 5-substituted analogs of 2'-deoxypyrimidine nucleosides and of three reference compounds (ara-C, IUdR and AIU) on the replication of herpes simplex virus type 1 (HSV-1), strain V3, grown under one-step conditions on monolayers of primary human lung fibroblast (PHLF) cultures, was determined (Table 1). At the concentration of 100 μM only the antiviral activities of BrVUdR, AMeUdR, fUdR, and HMeUdR were comparable with those of ara-C and IUdR. The reduction of virus yields by MMeUdR and HMeCdR as well as by BOMeUdR was lower but still similar to that given by AIU. The other derivatives investigated showed little or no activity against HSV-1, even at concentrations of 500–1000 μM. Differences between the most potent compounds (the first six in Table 1) in their ability to reduce one-step virus yields became apparent at low concentrations (1–10 μM). At these concentrations the following order of decreasing activity was noted: BrVUdR > ara-C > IUdR > AMeUdR > fUdR > HMeUdR. The difference between 1 μM IUdR and ara-C was significant at the 5% level, whereas the difference between 100 μM HMeCdR and BOMeUdR was significant at the 1% level (see Table 1).

In a second set of experiments all compounds were screened for activity against HSV-1-V3 in a multistep virus replication assay (plaque reduction test) on Vero cells (Table 1). The most effective compounds were BrVUdR and ara-C followed by HMeUdR, fUdR, IUdR and HMeCdR. There were considerable changes in the potency of the compounds. Whereas ara-C, HMeUdR, HMeCdR, and BOMeUdR were almost inactive in the

TABLE 1

Inhibition of HSV-1-V3 one-step replication and of plaque formation by 5-substituted 2'-deoxypyrimidine nucleosides

Compound	Log reduction in p.f.u./0.1 ml culture Concentration of analogs (μM)						Concentration of compounds (μM) for 50%/99% plaque reduction
	500	100	10	1	0.5	0.1	
5-(2-Bromovinyl)-UdR (BrVUdR)		3.2	3.2	3.2	1.9	0.4	0.2/ 0.6
Cytosine arabinoside (ara-C)		2.8	2.8	1.8	0.4	0.2	0.2/ .06
5-Iodo-UdR (IUdR)		3.0	2.9	1.1	0.2		4.6/ 10
5-Azidomethyl-UdR (AMeUdR)		2.8	2.1	0.1			36 / 100
5-Formyl-UdR (fUdR)		2.8	1.2	0.2			3.6/ 10
5-Hydroxymethyl-UdR (HMeUdR)		2.5	0.3				1.8/ 7.0
5-Iodo-5'-amino-2',5'-di-UdR (AIU)	2.9	1.4	0.0				700 /2000
5-Mercaptomethyl-UdR (MMeUdR)	2.2	1.6	0.0				800 /2500
5-Hydroxymethyl-CdR (HMeCdR)	2.3	1.4	0.0				7.0/ 23
5-Benzyloxymethyl-UdR (BOMeUdR)	1.4	0.9	0.0				17 / 50
5-Cyanomethyl-UdR	0.6	0.5					> 1000
5-Benzylaminomethyl-UdR	0.5	0.2					> 1000
5-Thiosemicarbazone of fUdR	0.5	0.1					220 / 500
5-Thiocyanomethyl-UdR	0.5	0.1					> 1000
5-Ethylmercaptomethyl-UdR	1.1[a]	0.0					> 1000
5-Ethyleneiminomethyl-UdR	0.2[a]	0.1					> 1000
5-Tertiary-butoxymethyl-UdR	0.2[a]	0.1					> 1000
5-Dinitrophenylhydrazone of fUdR		0.0[b]					240 / 900

[a] At 1000 μM.

[b] Limit of solubility.

one-step replication assay at 0.5 μM (ara-C) or 10 μM (HMeUdR, HMeCdR, and BOMeUdR) (Table 1), at similar concentrations they inhibited plaque formation by 99% (see also Table 1). In contrast, substances such as AMeUdR, MMeUdR and AIU, which showed a strong activity (two log reductions) in the one-step replication assays at 10, 250 and 250 μM respectively, were without any inhibitory effect against plaque formation at the same concentrations. MMeUdR and HMeCdR, showing a similar inhibition of virus replication in the one-step experiment, differed substantially in the plaque inhibition assay, MMeUdR being 100 times less effective than HMeCdR.

Similar inhibitory effects in both tests were demonstrated only by BrVUdR, fUdR and IUdR. BrVUdR was the most potent of the 5-substituted UdR derivatives and the only one that considerably surpassed the antiherpes potency of IUdR.

Spectrum of antiviral activity of BrVUdR

The influence of BrVUdR on the replication of some members of the herpes virus group, including HSV-2 strain US, pseudorabies virus, and four strains of HSV-1, (F, V3, Cl 101, and Kupka), was estimated by reduction of plaque formation on Vero cells and, additionally, on BHK 21/C13 cells for pseudorabies virus.

As can be seen from Table 2, the ID_{50}s for the HSV-1 strains varied from 0.06 to 0.22 μM with the highest ID_{50} value (0.22 μM) for HSV-1-V3. The importance of the cell line chosen for the plaque inhibition assay is obvious when we compare BrVUdR inhibition of pseudorabies virus on BHK 21/C13 cells (ID_{50} = 0.02 μM) with that on Vero cells (ID_{50} = 0.23 μM). HSV-2-US (ID_{50} = 8 μM) behaved quite differently from the HSV-1

TABLE 2

Activity of 5-(2-bromovinyl)-UdR against various DNA and RNA viruses

Virus/cell system	Concentration (μM) required to reduce the number of plaques by 50%
Vaccinia virus/BHK 21/C13 and Vero	> 500
Adenovirus type 1/Vero	> 100
Adenovirus type 2/Vero	100
Adenovirus type 5/Vero	50–100
Herpes simplex virus type 1/Vero	0.06–0.22[a]
Herpes simplex virus type 2/Vero	8
Pseudorabies virus/BHK 21/C13	0.02
Pseudorabies virus/Vero	0.23
Sindbis virus/BHK 21/C13 and Vero	> 500
Vesicular stomatitis virus/BHK 21/C13 and Vero	> 500
Fowl plague virus/CEF	> 100

[a] Strain F, V3, Cl 101, and Kupka.

strains, since it required an approximately 100-fold higher drug concentration for inhibiting virus replication. BrVUdR was virtually inactive against vaccinia virus and adenovirus type 1 and had little effect on adenoviruses type 2 and type 5 at concentrations inhibiting the proliferation of Vero cells by 50%. As could be anticipated, BrVUdR was totally inactive against vesicular stomatitis virus, Sindbis virus and fowl plague virus.

Inhibition of cell growth, reversibility of drug action and antiviral index of some 5-substituted pyrimidine nucleoside analogs

The influence of a number of 5-substituted 2′-deoxypyrimidine nucleosides on the growth of synchronized BHK 21/C13/2P suspension cells was assessed (Table 3). Of the tested compounds, BrVUdR proved the least cytostatic (ID_{50} = 100 μM). Even at 250 μM BrVUdR, synchronized BHK 21 populations (beginning in G_1) proceeded through a complete cell cycle. Following mitosis cells remained vital even for a period of 56 h (three generations for untreated cell cultures). The same is true for Vero cells (ID_{50} = 90 μM) and primary human lung fibroblast cultures which did not contain an appreciable amount of dead cells (< 5%) even when exposed to 100 μM BrVUdR for 4 and 5–6 days, respectively.

To determine the reversibility of BrVUdR action, G_1 populations of BHK 21 cells which had been treated with BrVUdR for 65 h (1, 10, and 50 μM) were washed (cf. Materials and Methods) and diluted to the cell density of the control culture and cell growth was followed for another 42 h (three generations).

After removal of the compound, cell growth was almost completely unaffected. An enhanced proliferation observed, in the treated cultures in the first two generations after drug removal, was probably due to a synchronizing effect of the compound resulting in

TABLE 3

Antiherpes and cytostatic activity of some 5-substituted 2′-deoxypyrimidine nucleosides

Compound	ID_{50} (μM) virus	ID_{50} (μM) cell[a]	Antiviral index[b] (*I*)
5-(2-Bromovinyl)-UdR	0.1[c]	100	1000
5-Hydroxymethyl-CdR	7	70	10
5-Iodo-UdR	4.6	20	4
5-Formyl-UdR	3.6	4	1.1
5-Benzyloxymethyl-UdR	17	8	0.5
5-Hydroxymethyl-UdR	1.8	0.2	0.1

[a] Concentration required to inhibit proliferation of BHK 21/C13/2P suspension cells by 50% (for details see Materials and Methods).

[b] Determined by dividing the ID_{50} for BHK 21/C13/2P cells by the ID_{50} for HSV-1-V3.

[c] Mean value of four HSV-1 strains.

the collection of cells in the later phases of the cell cycle thus being nearer mitosis than the control cells.

The antiviral indices (ratio of ID_{50} for cells to ID_{50} for virus (see Table 3)) reveal extreme differences in the antiviral selectivity of 5-substituted 2′-deoxypyrimidine nucleosides. The selectivity of BrVUdR (I = 1000) surpassed that of all the other analogs, of which only IUdR and HMeCdR displayed a certain selectivity, which fUdR, BOMeUdR and HMeUdR inhibited non-infected cells equally well or 2–10-fold more strongly than virus replication.

DISCUSSION

On the basis of their structure–activity relationship as published here and elsewhere, the 5-substituted 2′-deoxypyrimidine nucleosides may be divided into three different groups:

1. Compounds with a small 5-substituent which sterically and electronically resembles the methyl group (i.e. thymidine analogs). These compounds show a high antiherpes virus activity in both the single and multiple virus-growth cycle assays. This applies to UdR analogs like 5-hydroxymethyl-UdR (HMeUdR), 5-formyl-UdR (fUdR), and 5-mercaptomethyl-UdR (MMeUdR) (Table 1) as well as to others mentioned in the literature: 5-halogeno-UdR analogs and 5-trifluoromethyl-UdR [15, 30]; 5-ethyl-UdR [3, 16]; 5-vinyl-UdR [3, 8]; 5-ethynyl- and 5-(1-chlorovinyl)-UdR [10]; 5-hydroxy-UdR [32]; 5-methylmercapto-UdR [21]; 5-nitro-UdR [10]. All these analogs may be so similar to 2′-deoxythymidine that they are metabolized by viral and host cell enzymes (i.e. deoxythymidine kinase) with equal efficiency and are therefore, with only few exceptions, not selective.

2. Compounds with more than three side chain atoms of whatever type (C, O, S, N, halogen) and/or a more bulky group. We found that these are only marginally active or totally inactive (see Table 1). Only 5-azidomethyl-UdR (AMeUdR) and 5-benzyloxymethyl-UdR (BOMeUdR) were somewhat active against HSV-1, be it 8- or 4-fold less so than IUdR (Table 1). The BOMeUdR data were surprising, since Babiuk and his coworkers [1, 27] reported no effect of this substance on HSV-1 replication in HEp-2 cells. Of the known 5-XUdR analogs described in the literature a large number of 5-alkyloxymethyl-UdR [27], 5-alkyloxy-UdR [32], 5-alkylaminomethyl-UdR and 5-halogenoacetamidomethyl-UdR analogs [12], with the exception of 5-propynyloxy-UdR [32], were all inactive. It seems that these compounds are not normally accepted either by viral or cellular enzymes, but if they are metabolized, as 5-azidomethyl-UdR (Table 1) and 5-propynyloxy-UdR [32] are, then they also show selectivity in their antiviral action.

3. Compounds with three side chain atoms. About all of these possess a high activity and a more or less pronounced selectivity, as shown by 5-propyl-UdR [3, 13] and, above all, BrVUdR (see Table 3) as well as 5-(2-chloro- and -iodovinyl)-UdR [11] which presently meet the optimal criteria for anti-herpes activity and selectivity. The crucial role of the size of the 5-substituent in antiviral activity and selectivity is evident from the

biological properties of 5-XUdR derivatives with the side chain $-CH_2-S-R$ or $-CH_2-O-R$. In the case of R = H the two compounds, MMeUdR and HMeUdR, are both antiviral (Table 1) [20, 34] and cytostatic (Table 3) [23, 33], i.e. not selective. In contrast, when R = CH_3 the resulting compounds, 5-methylthiomethyl-UdR [5, 7, 12] and 5-methoxymethyl-UdR [1], are efficient and selective as antiherpetic compounds, but when R = C_2H_5 the analogs are neither cytostatic nor antiviral (Table 1) [23, 27].

This shows that the antiviral activity and selectivity pass through an optimum with respect to the size and electronic properties of the 5-substituent. Outside this optimum the compounds are either not selective or not active at all. Some of the exceptions found in the three groups are characterized by substituents with double or triple bonds which may confer special spatial and electronic properties.

The importance of the test system for the evaluation of potentially active antiviral compounds is evident from a comparison of the antiherpetic activities of 5-substituted 2′-deoxypyrimidine nucleosides in one-step experiments with those obtained in the plaque reduction assay (Table 1). Most compounds are more effective in the plaque reduction assay and only three, namely AMeUdR, AIU and MMeUdR, act more strongly in the one-step assay. At least for MMeUdR this could be due to a chemical modification during the prolonged treatment period (formation of an inactive disulfide product [21]). For compounds such as HMeUdR (that afford complete inhibition of plaque formation at 10 μM, without any effect in the one-step assay), the stronger inhibition in the multi-step assay may be due to the incorporation of HMeUdR into viral DNA. This incorporation may not affect the first but all the subsequent virus growth cycles as a result of accumulation of the analog. Similar effects have been reported with HMeUdR [25] and 5-vinyl-UdR [3, 22] in Ehrlich's ascites tumor cells. The repetitive virus replication cycle corresponds more closely to the natural situation than the one-step virus replication cycle. Hence the plaque reduction assay should be the test of choice for the evaluation of antiviral agents.

BrVUdR is much more potent than IUdR in both the single- and multiple-step replication assay and also more potent than ara-C in the one-step assay. Remarkable is also the very low antiproliferative effect of BrVUdR. We found that even after prolonged treatment the inhibitory effect of BrVUdR on cell proliferation is fully reversible. Our results confirm those of De Clercq et al. [5, 12, 14] who were the first to establish the efficiency and selectivity of BrVUdR as an anti-HSV-1 agent. For Vero cells the antiviral ID_{50} of BrVUdR was 3–10 times higher than for primary rabbit kidney cells (0.02 μM) (see Tables 1 and 2). However, as shown in Table 2, the ID_{50} of BrVUdR for pseudorabies virus was approximately one order of magnitude lower in BHK 21/C13 cells (0.02 μM) than in Vero cells (0.23 μM). Similarly, the ID_{50} values of BrVUdR for a series of HSV-1 laboratory strains and clinical isolates (J. Reefschläger, P. Wutzler et al., manuscript in preparation) were about 10 times lower in the plaque inhibition assay on primary rabbit testes (RTP) cells (ID_{50} = 0.01–0.02 μM) than on Vero cells (ID_{50} = 0.06–0.22 μM).

The potency of BrVUdR against HSV-1 does not only surpass that of IUdR and ara-C (cf. Table 1) but is also superior to that of 9-(2-hydroxyethoxymethyl)guanine (acyclo-G)

[19, 29], 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC) [35], 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-methyluracil (FMAU) [35], and 9-β-D-arabinofuranosyladenine (ara-A) which were compared for inhibiting HSV-1 plaque formation in two different cell lines (Vero and BHK 21/C13 cells) (J. Reefschläger, manuscript in preparation). At about a 100-fold higher concentration than required for inhibiting HSV-1 plaque formation, BrVUdR also inhibited HSV-2 plaque formation (ID_{50} = 8 μM in Vero cells) (Table 2), and a number of HSV-2 laboratory strains and clinical isolates plated on RTP cells were only inhibited at 400–2500 higher BrVUdR concentrations (ID_{50} = 8–25 μM) than HSV-1 (J. Reefschläger, P. Wutzler et al., manuscript in preparation). The remarkable difference in sensitivity of HSV-1 and HSV-2 towards BrVUdR indicates that BrVUdR may be useful as a marker test for identifying HSV-1 and HSV-2 in clinical isolates, as has originally been proposed by De Clercq et al. [14].

It was not surprising to find that BrVUdR inhibited the replication of an animal herpes virus, pseudorabies virus (PRV), to the same degree as HSV-1 (cf. Table 2), since, without exception, the antiviral effects of all 5-XUdR and 5-XCdR analogs versus HSV-1 coincided with those versus PRV (data not shown).

The relatively simple chemical synthesis of BrVUdR, its strong activity against HSV-1 in cell culture and the reversibility of the slight inhibitory action on cell growth at high drug concentrations, as well as our results with BrVUdR in the treatment of experimental herpes encephalitis in mice (J. Reefschläger, P. Wutzler et al., manuscript in preparation) and favourable results obtained with BrVUdR by other investigators in animal experiments [9, 18, 26], make BrVUdR a promising substance for the treatment of herpes simplex virus type 1 infections in humans.

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