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Antiherpes activity of (*E*)-5-(2-bromovinyl)- and 5-vinyl-1-β-D-arabinofuranosyluracil and some other 5-substituted uracil arabinosyl nucleosides in two different cell lines*

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Of a series of 5-substituted 1-β-D-arabinofuranosyluracil (5-*X*-araU) analogues, (*E*)-5-(2-bromovinyl)-araU(BrVaraU) and 5-vinyl-araU (VaraU) were the most potent inhibitors of plaque formation by two herpes simplex virus type 1 (HSV-1) strains in human embryonic lung fibroblast (HELF) cell cultures. They were not only more active than 5-methyl-araU (MaraU, araT) and 5-ethyl-araU (EaraU), but even more than 1000 times more potent than the 5-fluoro, 5-iodo, 5-formyl and 5-trifluoromethyl (FaraU, IaraU, faraU, CF_3araU) analogues. BrVaraU and VaraU were superior to 9-(2-hydroxyethoxymethyl)guanine (Acyclovir, ACV) and comparable in potency with 2'-fluoro-5-iodoaracytosine (FIAC) and 2'-fluoro-5-methylarauracil (FMAU). Their anti-HSV-1 potency was surpassed only by (*E*)-5-(2-bromovinyl)-2'-deoxyuridine (BrVUdR). Surprisingly, in a HSV-1 plaque inhibition assay in African green monkey kidney (Vero) cells, BrVaraU and VaraU were nearly 100 times less active or even inactive. In contrast, the antiherpes activity of ACV, FIAC, FMAU and BrVUdR differed only marginally in the two cell lines. The following order of (decreasing) activity against HSV-2 in HELF cells was found: FIAC = FMAU > MaraU (araT) > ACV > VaraU > BrVUdR > CF_3araU > IaraU > FaraU = EaraU > BrVaraU > araU > faraU. When deoxyribose is replaced by arabinose in 5-*X*-UdR analogues, a slight increase in anti-HSV-1-77 activity was observed for the 5-vinyl or 5-ethyl substituent, whereas the other 5-*X*-araU nucleosides were two to more than 100 times less active than their deoxyribosyl counterparts. However, the sugar exchange led to a strong reduction in anti-HSV-2 activity regardless of the 5-substituent.

herpes simplex virus strains; 5-substituted 1-β-D-arabinofuranosyluracils; human embryonic lung fibroblasts; African green monkey kidney (Vero) cells; 5-formyl-1-β-D-arabinofuranosyluracil; 5-fluoro-1-β-D-arabinofuranosyluracil; 5-trifluoromethyl-1-β-D-arabinofuranosyluracil

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Introduction

Among the nucleoside analogues synthesized so far, (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BrVUdR) is one of the most potent inhibitors of herpes simplex virus type 1 (HSV-1) [8,32] and varicella-zoster virus (VZV) [16]. It is, however, 100–2500 times less active against herpes simplex virus type 2 (HSV-2) [10,33]. All attempts to overcome this deficiency by modifying the 5-(2-*X*-vinyl) [11,12, J. Reefschläger et al., submitted] or the sugar [15] substituent have been in vain. If active at all, these derivatives displayed the same 'activity gap' between HSV-1 and HSV-2 as did BrVUdR or, if not, they were much less efficient (J. Reefschläger et al., submitted). On the other hand, certain 5-substituted 1-β-D-arabinofuranosyluracil (araU) and -cytosine (araC) nucleosides like 5-methyl-araU (1-β-D-arabinofuranosylthymine, araT), 2′-fluoro-5-iodo-araC (FIAC) and 2′-fluoro-5-methyl-araU (FMAU) are selective and potent inhibitors of both HSV-1 and HSV-2 [18,22,23,40]. A number of 5-substituted UdR analogues, 5-vinyl-, 5-formyl-, 5-iodo-, 5-fluoro- and 5-trifluoromethyl-UdR also inhibit HSV-1 and HSV-2 equally well; however, with the exception of 5-vinyl-UdR they do so at higher concentrations than the 5-substituted arabinosyl nucleosides [9–11,21,32, J. Reefschläger et al., submitted].

Recently, procedures for synthesizing (*E*)-5-(2-bromovinyl)-araU (BrVaraU [3,34]) and 5-vinyl-araU (VaraU) [34] have been described and biological data for these compounds [15,17,24–29) and for 5-ethyl-araU (EaraU) [19,23] and 5-iodo-araU (IaraU) [35,40] were published.

We have also synthesized several new 5-substituted araU analogues including BrVaraU and VaraU as well as 5-formyl-, 5-fluoro- and 5-trifluoromethyl-araU (faraU, FaraU, CF_3araU) and have examined their effects on the plaque formation of two HSV-1 strains in two different cell lines and of HSV-2. We find that, as inhibitors of HSV-1 replication in human embryonic lung fibroblasts (HELF), BrVaraU and VaraU are superior to all previously studied 5-substituted araU analogues and 9-(2-hydroxyethoxymethyl)guanine (Acyclovir, ACV) and comparable to FIAC and FMAU. However, they are even less active against HSV-2 than the corresponding UdR analogues.

Surprisingly, the 5-substituted araU analogues are markedly less effective or even ineffective (BrVaraU, VaraU) in African green monkey kidney (Vero) cells. For BrVaraU this observation has already been made by De Clercq [14]. In contrast, the corresponding 5-substituted UdR analogues, FIAC, FMAU and ACV retain their full antiherpes activity in Vero cells. This is an important finding, since Vero cells are routinely used in many laboratories to evaluate the antiviral properties of new compounds.

Materials and methods

Chemicals

5-Iodo-2′-deoxyuridine (IUdR) and 5-fluoro-2′-deoxyuridine (FUdR) were from

Serva (Heidelberg, F.R.G.); araU was from Calbiochem (San Diego, CA, U.S.A.). FIAC and FMAU were a kind gift of Dr. J.J. Fox (Sloan-Kettering Institute, NY, U.S.A.); ACV was kindly provided by Burroughs Wellcome Co. (Research Triangle Park, NC, U.S.A.). 5-Ethyl-2'-deoxyuridine (EUdR) was prepared by the method of Niedballa and Vorbrüggen [31]. BrVUdR as well as 5-vinyl- and 5-formyl-UdR (VUdR, fUdR) were prepared as described previously [2,20,32]. AraT and EaraU were prepared by the method of Nakayama et al. [30], IaraU by the method of Schinazi et al. [35] and FaraU as well as CF_3araU were synthesized as described elsewhere [4,36,37].

Synthesis of nucleoside analogues

5-Formyl-1-β-D-arabinofuranosyluracil This compound was prepared by conversion of 1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)thymine [30] analogues to the method described previously [2] (m.p. 222–223°C, orange crystals; UV (MeOH): λ_{max} 282 nm, 237 nm; MS: m/e, 272 (M^+); PNR data (DMSO-d_6): $\delta = 9.69$ (1 H, S; 5-CHO), $\delta =$ 8.27 (1 H, S;, 6-H)).

5-Vinyl- and (E)-5-(2-bromovinyl)-1-β-D-arabinofuranosyluracil 1.90 g (4.77 mmol) of 5-ethyl-1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)uracil was brominated in chloroform solution under irradiation. After HBr elimination with triethylamine the reaction mixture was chromatographed on silica gel: (*E*)-5-(2-bromovinyl)-1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)uracil (815 mg, 36%, sirup); 5-vinyl-1-(2,3,5-tri-*O*-acetyl-β-D-arabinofuranosyl)uracil (567 mg. 30%, m.p. 146–148°C (EtOH)). Deblocking with 0.1 N methanolic sodium methylate solution yielded the nucleosides. (*E*)-5-(2-Bromovinyl)-1-β-D-arabinofuranosyluracil: m.p. 182–184°C (EtOH), compared with 182°C and 179–181°C found by Sakata et al. [34] and Busson et al. [3], respectively; UV (MeOH): λ_{max} 253, 295 nm. 5-Vinyl-1-β-D-arabinofuranosyluracil: m.p. 196–200°C (EtOH); UV (MeOH): λ_{max} 241, 292 nm. The structure of the compounds was established by mass spectroscopy, NMR and elemental analyses.

Cells and viruses

The origin of HELF and of Vero cells, details of their cultivation and media, the origin of the recent clinical isolates HSV-1-77, HSV-2-82 and HSV-2-42/78 as well as the laboratory strain HSV-1-V3 and the plaque inhibition assay in Vero cells have been described previously [32,33]. Plaque inhibition assays using HELF cell cultures were performed in 50-ml culture flasks with nearly confluent monolayers, 24 h after seeding. Cells were infected with 0.2 ml of a virus suspension yielding 50–100 plaques per bottle. After a 1-h virus adsorption period 3.6 ml of a methocel (0.5% w/v) overlay medium containing 10% fetal calf serum and 0.2 ml of appropriate substance solutions were added to each culture. The following procedure and analysis were the same as previously published [32]. The ID_{50} values are the means of 2–6 plaque inhibition assays performed with three concentrations each within the inhibitory range of the compounds and with triplicate cultures. The mean inhibition values for these three concentrations were plotted on a decimal scale against the logarithm of the concentra-

tion, and the concentration inhibiting plaque formation by 50% was determined graphically.

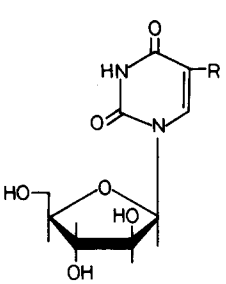
Results

Inhibition of HSV-1 in human embryonic lung fibroblast (HELF) cell cultures

The inhibition of HSV-1-77 and HSV-1-V3 plaque formation in HELF cells by a number of 5-substituted araU derivatives (Fig. 1) and by the reference compounds ACV, FIAC, FMAU and BrVUdR is shown in Table 1. BrVaraU and VaraU were the most effective inhibitors of HSV-1-77. They were superior to all other 5-substituted araU nucleosides including MaraU (araT) and the reference compounds ACV, FIAC and FMAU and surpassed only by BrVUdR. The parent compound araU as well as faraU and the 5-halogeno derivatives (IaraU, FaraU, CF_3araU) were about 1000 times less effective than the two 5-alkenyl derivatives. Towards HSV-1-V3 BrVaraU and VaraU were also highly active, more so than ACV, but less active than FIAC, FMAU and BrVUdR. AraU, faraU and, surprisingly, even EaraU were totally ineffective up to a concentration of 1000 μM. From the ratios of the anti-HSV-1-V3 for the anti-HSV-1-77 effects (Table 1, column 4) it is clear that FIAC, FMAU and BrVUdR are similarly effective against both strains, whereas VaraU, BrVaraU, araT, ACV and EaraU are 5 to more than 476 times less effective against HSV-1-V3.

Inhibition of HSV-1 in African green monkey kidney (Vero) cells

The sensitivity of HSV-1-77 and HSV-1-V3 towards 5-substituted araU analogues and the reference compounds was also tested by the plaque inhibition assay in Vero cells. Here BrVaraU, VaraU and araT were similarly active against HSV-1-77, yet 5–65 times less than FMAU, ACV, FIAC and BrVUdR, and 18–84 times less active than in HELF cell cultures. In contrast, the reference compounds showed only slight differences in both cell lines (Table 2, columns 2 and 3). The reduction of the anti-herpes potency of BrVaraU and VaraU in Vero cells is especially manifest in the case of HSV-1-V3 (Table 2, columns 4 and 5). Again, the reference compounds were



R=	
-CH=CHBr	(E)-5-(2-bromovinyl)-araU
$-CH=CH_2$	5-vinyl-ara U
$-CH_3$	5-methyl-ara U(ara T)
$-CH_2-CH_3$	5-ethyl-ara U
-F	5-fluoro-ara U
-I	5-iodo-ara U
-CHO	5-formyl-ara U
-H	ara U
$-CF_3$	5-trifluoromethyl-ara U

Fig. 1. Structural formulae of 5-substituted 1-β-D-arabinofuranosyluracil (araU) analogues.

TABLE 1

Inhibitory effects of 5-substituted araU analogues and some reference compounds on plaque formation by two HSV-1 strains in HELF cell cultures

Compound	ID_{50} (μM)[a]		Ratio $\frac{ID_{50}\ (HSV\text{-}1\text{-}V3)}{ID_{50}\ (HSV\text{-}1\text{-}77)}$
	HSV-1-77	HSV-1-V3	
(*E*)-5-(2-Bromovinyl)-araU(BrVaraU)	0.045	0.55	12
5-Vinyl-araU (VaraU)	0.08	0.37	5
5-Methyl-araU (MaraU, araT)	0.24	3.1	13
5-Ethyl-araU (EaraU)	2.1	>1000	>476
5-Fluoro-araU (FaraU)	55	-	-
5-Iodo-araU (IaraU)	70	-	-
5-Formyl-araU (faraU)	80	>500	>6
5-Trifluoromethyl-araU (CF_3araU)	85	-	-
araU	120	>1000	>8
ACV	0.24	6.50	27
FIAC	0.14	0.12	1
FMAU	0.26	0.09	<1
BrVUdR	0.026	0.05	2

[a] ID_{50} = concentration required to reduce the number of plaques by 50% compared with an untreated infected control culture.

TABLE 2

Inhibitory effects of 5-substituted araU analogues and some reference compounds on plaque formation by two HSV-1 strains in Vero cell cultures

Compound	HSV-1-77		HSV-1-V3		Ratio $\frac{ID_{50}\ (HSV\text{-}1\text{-}V3)}{ID_{50}\ (HSV\text{-}1\text{-}77)}$
	ID_{50} (μM)	Ratio $\frac{ID_{50}\ (Vero)}{ID_{50}\ (HELF)^*}$	ID_{50} (μM)	Ratio $\frac{ID_{50}\ (Vero)}{ID_{50}\ (HELF)^a}$	
BrVaraU	3.8	84	>1000	>1818	>263
VaraU	3.2	40	1000	2702	313
MaraU (araT)	4.2	18	57	18	14
EaraU	23	11	>1000	1	>44
faraU	460	6	>1000	2	>2
araU	>1000	>8	>1000	1	1
ACV	0.41	2	15	3	37
FIAC	0.12	1	0.25	2	2
FMAU	0.65	3	0.85	9	1
BrVUdR	0.065	3	0.23	5	4

[a] Data from Table 1.

almost as active as expected but the effect of araT was diminished and BrVaraU and VaraU were virtually inactive. Little differences were noted in the ID_{50} vs HSV-1-V3 and ID_{50} vs HSV-1-77 for FIAC, FMAU and BrVUdR. However, for araT, ACV, EaraU, VaraU and BrVaraU (Table 2, column 6) significant differences were found.

Inhibition of HSV-2 in HELF cell cultures

None of the new 5-substituted araU derivatives was superior as an inhibitor of HSV-2-42/78 to araT and ACV, FIAC and FMAU, and only VaraU was more effective than BrVUdR (Table 3). The following order of decreasing activity was found for the active compounds: FIAC = FMAU > araT > ACV > VaraU. Whereas the reference compounds FIAC, FMAU and araT differed only slightly in their inhibitory activity against HSV-1 and HSV-2 (Table 3, column 3) the other active HSV-1 inhibitors ACV and EaraU, as well as the two 5-*X*-araU analogues VaraU and BrVaraU and the reference substance BrVUdR, showed a 10- to > 1000-fold lower activity against HSV-2.

Comparison of 5-substituted araU and the corresponding 5-substituted UdR analogues in their activity against HSV-1 and HSV-2

With the exception of VaraU and EaraU, the other 5-substituted araU derivatives were less active against HSV-1-77 than the corresponding 5-substituted UdR analogues (Table 4, column 6). VaraU was also more effective than VUdR against HSV-1-V3: ratio ID_{50} (araU)/ID_{50} (UdR) = 0.7. For EaraU (ID_{50} vs HSV-1-V3 > 1000 μM), as

TABLE 3

Inhibitory effects of 5-substituted araU analogues and some reference compounds on plaque formation by HSV-2-42/78 in HELF cell cultures

Compound	ID_{50} (μM)	Ratio $\frac{ID_{50}\text{ (HSV-2-42/78)}}{ID_{50}\text{ (HSV-1-77)}^a}$
BrVaraU	290	6444
VaraU	9	113
MaraU (araT)	1.4	6
EaraU	180	86
FaraU	156	3
IaraU	100	1
faraU	>500	>6
CF_3araU	72[b]	1[b]
araU	440	4
ACV	3.4	14
FIAC	0.37	3
FMAU	0.48	2
BrVUdR	27	1039

[a] Data from Table 1.

[b] For HSV-2-82.

TABLE 4

Inhibitory effects of some 5-substituted araU analogues and their corresponding 5-substituted UdR analogues on plaque formation by HSV-1 and HSV-2 in HELF cell cultures

5-Substituent	ID_{50} (μM)				Ratios			
	HSV-1-77		HSV-2-42/78		$\frac{ID_{50}\text{ (araU)}}{ID_{50}\text{ (UdR)}}$		$\frac{ID_{50}\text{ (HSV-2-42/78)}}{ID_{50}\text{ (HSV-1-77)}}$	
	araU[a]	UdR	araU[b]	UdR	HSV-1-77	HSV-2-42/78	araU[b]	UdR
E-CH=CHBr	0.045	0.026	290	27	2	11	6444	1039
$-CH=CH_2$	0.08	0.26	9	2.2	0.3	4	113	9
$-CH_2-CH_3$	2.1	10	180	7	0.2	26	86	<1
-F	55	0.3	156	3.7	183	42	3	12
-I	70	0.8	100	2.2	88	46	1	3
-CHO	80	6.8	>500	6.0	12	>83	>6	1

[a] Data from Table 1.

[b] Data from Table 3.

compared to EUdR (ID_{50} = 11 μM), the ratio was > 90. Compared with their UdR counterparts the araU analogues were also 4 to > 83 times less potent inhibitors of the replication of HSV-2-42/78 (Table 4, column 7). For the active araU analogues BrVaraU, VaraU and EaraU (Table 4, columns 8 and 9) the substitution of arabinose for deoxyribose enlarged the 'gap' between anti-HSV-1- and anti-HSV-2-activity.

Discussion

Our results show that, of all 5-substituted araU (5-*X*-araU) derivatives so far tested, BrVaraU and VaraU were the most potent inhibitors of HSV-1 replication in vitro (Table 1), thus confirming the results of Machida et al. [25] and DeClercq et al. [15]. Towards a clinical isolate (HSV-1-77) BrVaraU and VaraU were also more active than FIAC, FMAU and ACV; only BrVUdR was 3–6 times stronger (Table 1). As Machida et al. [23] reported previously, the anti-HSV-1 activity of the araU derivatives declined sharply with increasing the chain length of the 5-alkyl substituent. Thus, EaraU was 10 times less active than araT [19] (Table 1). With the 5-substituted UdR (5-*X*-UdR) analogues (whereby *X* = ethyl, propyl), substitution of *X* by an alkenyl group (vinyl, propenyl) or substitution of one of the H atoms of the vinyl C-2 by a halogen (Cl, Br, I) yields more effective compounds [5,6,8,11,32,38, J. Reefschläger et al., submitted] (Table 4, column 3). Similarly, the anti-HSV-1 activity of the 5-*X*-araU analogues increases in the order ethyl < vinyl < 2-bromovinyl (Table 1). Surprisingly, and in contrast with the 5-*X*-UdR analogues, FaraU, IaraU, CF_3araU, faraU and araU were 200–400 times less active than araT and 1000 times less than BrVaraU and VaraU (Table 1). Only few 5-*X*-araU analogues are known as highly active antiherpes compounds: e.g. BrVaraU, (*E*)-5-(2-chlorovinyl)-araU, VaraU, araT and EaraU. Other 5-*X*-araU analogues show little, if any, antiviral activity [7,13,23,24,34,35,39]. Among these are compounds with those 5-substituents that in the UdR series result in active inhibitors of herpesvirus replication: e.g. 5-methoxymethyl-, 5-propynyloxy-, 5-acetonyl-, 5-propyl-, and 5-(1-propenyl)-araU. We could not confirm the claim [35] that IaraU is a potent antiviral substance; it is in fact 300 times less active than araT (Table 1). The laboratory strain HSV-1-V3 was considerably less sensitive to the 5-substituted araU analogues and to ACV than the clinical isolate HSV-1-77; it was completely refractory to EaraU, yet equally sensitive as HSV-1-77 to FIAC, FMAU and BrVUdR (Table 1) and other 5-substituted 2'-deoxyuridine analogues (J. Reefschläger et al., submitted). Machida et al. [23] found that a HSV-1 isolate from a herpes keratitis patient was hardly sensitive to araT, EaraU and EUdR, while another patient's isolate was effectively inhibited by these compounds. They suggested that the substrate specificity of HSV-1 pyrimidine deoxynucleoside kinase varies from one strain to another. Our results tend to support this concept and further indicate that the substrate specificities of the metabolizing enzymes for the sugar substituent (araU or UdR) and the substituent in 5-position (methyl or ethyl) may vary independently. On the one hand, the sensitivity of HSV-1-77 towards EaraU and EUdR differs only 5 times (Table 4), while EUdR (ID_{50} = 11 μM) is 100 times more active against HSV-1-V3 than EaraU (ID_{50} > 1000 μM; Table 1). On the other hand, 5-methyl-

araU and 5-ethyl-araU differ 10-fold in their activity against HSV-1-77 but more than 300-fold in their activity against HSV-1-V3. Similarly, MaraU and FMAU have the same effect on HSV-1-77 but differ 30-fold in their activity against HSV-1-V3. The fact that FIAC and FMAU, like 5-*X*-UdR derivatives (J. Reefschläger et al., submitted), hardly discriminate between the two HSV-1 strains may be explained by the deoxy character of the 2-fluoroarabinose, which is further attested by the cytostatic properties of the 2'-fluoro-araU analogues [40].

Obviously, the activity of 5-*X*-araU derivatives towards the relication of HSV-1 does not only depend on the virus strain but also on the host cell line (Table 2). The ID_{50} values recorded for plaque inhibition in Vero cells, especially with the 5-alkenyl compounds, are up to 100 times higher than in HELF cells, and in the case of HSV-1-V3 even 2000–3000 times higher. In contrast, the ID_{50} values of the reference compounds ACV, FIAC, FMAU and BrVUdR show little differences (Table 2). From the present data it is difficult to interpret the inactivity of BrVaraU and VaraU in Vero cells. As regards the anti-HSV-2 activity of the new 5-*X*-araU derivatives, none is more potent than araT, ACV, FIAC and FMAU, only VaraU was better than BrVUdR (Table 3). Our data thus support the weak anti-HSV-2 effect of BrVaraU observed by Machida et al. [25] and DeClercq et al. [15]. Apparently, the substitution of the C-2 atom in 5-vinyl-araU by bromine does not only enhance the effect on HSV-1 but concomitantly lowers the effect on HELF cell proliferation [25] and HSV-2 replication (Table 3). It seems that the HSV-2-specified enzyme(s) involved in nucleoside metabolism might have some similarities with the respective cellular enzymes. The replacement of deoxyribose by arabinose in the 5-*X*-UdR analogues led to a somewhat enhanced activity against HSV-1-77 only for 5-ethyl- and 5-vinyl-araU, while it lowered the activity of the other derivatives to a relatively small (BrVaraU) or great (faraU, IaraU, FaraU) extent (Table 4). (*E*)-5-(1-propenyl)-araU [24,38] has also a significantly lower anti-HSV-1 activity than the corresponding UdR derivative [6,11,38]. With respect to anti-HSV-2 activity, the substitution of arabinose for deoxyribose not only reduces the inhibitory potency (Table 4) but also magnifies the difference between the anti-HSV-1 and the anti-HSV-2-effect for most of the compounds (Table 4, columns 8 and 9), and especially for the active analogues BrVaraU, VaraU and EaraU. Only three compounds, namely FIAC, FMAU and araT, show a similar activity towards HSV-1 and HSV-2, and are also selective in their antiviral activity [1,8,23,40]. Their sugar component is arabinose or 2-fluoro-arabinose and their base is a uracil ring with a 5-methyl substituent (thymine) or cytosine with a 5-iodo substituent. Apparently, these features suffice to distinguish between the differences in the specificities of the HSV-2- and host-induced enzymes involved in nucleoside metabolism.

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