Antiviral Research, 1 (1981) 213–223
Elsevier/North-Holland Biomedical Press

ANTIHERPES ACTIVITY OF [E]-5-(1-PROPENYL)-2′-DEOXYURIDINE AND 5-(1-PROPENYL)-1-β-D-ARABINOFURANOSYLURACIL

G. STENING, B. GOTTHAMMAR, A. LARSSON, S. ALENIUS, N.G. JOHANSSON and B. ÖBERG

Department of Antiviral Chemotherapy, Research and Development Laboratories, ASTRA Läkemedel AB, Södertälje, Sweden

(Received 11 March 1981; accepted 17 June 1981)

5-(1-Propenyl)-1-β-D-arabinofuranosyluracil has been synthesized, and this compound and [E]-5-(1-propenyl)-2′-deoxyuridine have been tested for inhibition of herpes virus multiplication. Only [E]-5-(1-propenyl)-2′-deoxyuridine was found to be an active inhibitor reducing by 50% the plaque formation of herpes simplex virus type 1 (HSV-1) at about 1 μM. A comparison to the bromovinyl derivatives showed the following order of decreasing activity; [E]-5-(2-bromovinyl)-2′-deoxyuridine > 5-(2-bromovinyl)-1-β-D-arabinofuranosyluracil ≥ [E]-5-(1-propenyl)-2′-deoxyuridine > 5-(1-propenyl)-1-β-arabinofuranosyluracil. HSV-1 mutants lacking thymidine kinase or resistant against acycloguanosine were resistant against [E]-5-(1-propenyl)-2′-deoxyuridine. All compounds seemed to be phosphorylated by HSV-1 thymidine kinase in a cell-free assay. The compounds were phosphorylated to a lower extent by cellular or HSV-2 thymidine kinase, and the HSV-2 strains tested were inhibited by less than 50% at 100 μM in plaque assays. A selective inhibition of HSV-1 DNA synthesis by [E]-5-(1-propenyl)-2′-deoxyuridine was observed in infected cells indicating an effect on viral DNA polymerase. [E]-5-(1-Propenyl)-2′-deoxyuridine had a low cellular toxicity and a therapeutic effect when applied topically to HSV-1-infected guinea pig skin.

antiviral activity herpes simplex virus [E]-5-(1-propenyl)-2′-deoxyuridine 5-(1-propenyl)-1-β-D-arabinofuranosyluracil

INTRODUCTION

The first discovered useful antiherpes drug [15, 22] as well as the majority of the known inhibitors of herpes viruses are 5-substituted pyrimidine derivatives [6, 9, 24]. The general mechanism for their selective antiviral activity is a preferential phosphorylation by herpes virus-induced thymidine kinase [6, 9, 24]. Active inhibitors like [E]-5-(2-bromovinyl)-2′-deoxyuridine [7], 2′-fluoro-2′-deoxy-5-iodo-1-β-D-arabinofuranosylcytosine [26] and 5-ethyl-1-β-D-arabinosyluracil [16, 21] have provided the impetus to further explore these types of structures for improved anti-herpetic activity. The synthesis of [E]-5-(1-propenyl)-2′-deoxyuridine has been described recently [23], and biological data has been presented for this compound [3, 5] as well as for 5-(1-propenyl)-1-β-D-arabinofuranosyluracil [20]. We have synthesized 5-(1-propenyl)-1-β-D-

0166–3542/81/0000–0000/$02.50

arabinofuranosyluracil and investigated this compound and [E]-5-(1-propenyl)-2′-deoxyuridine [23] for anti-herpes activity and mode of action, and compared them to the structurally related bromovinyl derivatives. The antiherpes activity in cell culture showed the following order of decreasing activity: [E]-5-(2-bromovinyl)-2′-deoxyuridine, 5-(2-bromovinyl)-1-β-D-arabinofuranosyluracil, [E]-5-(1-propenyl)-2′-deoxyuridine (1-propenyl)-1-β-D-arabinofuranosyluracil.

MATERIALS AND METHODS

Isotopes and chemicals

Methyl [^{3}H] thymidine (49.5 Ci/mmol) was from New England Nuclear (Boston, MA) and ortho [^{32}P] phosphate (1 mCi/ml) from the Radiochemical Centre (Amersham, England). [E]-5-(2-Bromovinyl)-2′-deoxyuridine was a kind gift from Dr. E. De Clercq (The Rega Institute, Leuven, Belgium), and 5-(2-bromovinyl)-1-β-D-arabinofuranosyluracil was kindly given by Dr. H. Machida (Yamasa Shoyu Co., Ltd., Choshi, Chiba, Japan). Nucleoside triphosphates were from Sigma Co. (St. Louis, MO). 5-Propenyl nucleosides were synthesized as described in Synthesis of nucleoside analogues (p. 215). All chemicals were of analytical grade.

Thymidine kinase assay

Cell extracts from uninfected and from herpes simplex virus type 1 (HSV-1, strain C42) and type 2 (HSV-2, strain 91075) infected Vero cells were used as sources of thymidine kinase (TK) activity. The method was as described by Cheng and Ostrander [4] and by Lee and Cheng [19], but neither separation of a cytosol fraction nor purification on the affinity column was done. The assay mixture contained 150 mM Tris-HCl, pH 7.5, 10 mM ATP, 10 mM $MgCl_2$, 10 mM dithiothreitol, 5 μM methyl [^{3}H] thymidine. After 30 min of incubation at 37°C, 50 μl of the reaction mixture was added to Whatman DE 81 paper discs (2.3 cm) and washed twice with H_2O and three times with ethanol. The dried paper discs were counted in 3 ml Econoflour scintillation solution (New England Nuclear, Boston, MA). Phosphorylation of nucleoside analogues was determined as their ability to reduce the phosphorylation rate of [^{3}H] thymidine in the thymidine kinase assay. This method does not determine whether an analogue acts as an alternative substrate or as an inhibitor of the enzyme activity.

Cells and viruses

Vero cells (CCL 81) were grown as described earlier [11, 17] and used for plaque assays [11, 17]. Most of the herpes simplex virus strains used have been described earlier [11, 13]. An acycloguanosine [10]-resistant strain (HSV-1 C42 ACG^r) was selected by repeated passages of HSV-1 strain C42 in the presence of acycloguanosine. This resistant mutant was plaque-purified and propagated once in Vero cells in the absence of acycloguanosine.

Determination of HSV-1 and cellular DNA synthesis

The method to determine viral and cellular DNA synthesis by ortho[^{32}P]phosphate labelling and isodensity gradient centrifugation has been reported earlier [17, 18].

Cellular toxicity

The effect on cell proliferation was determined as described previously [25]. The test compound was added to actively growing Vero cells and incubated for 48 h. Cell numbers were measured by the use of an electronic cell counter (Analysinstrument AB, Stockholm) and the mean cell volume was also recorded. The results presented are means from two cell cultures.

Animal experiments

The method to inoculate guinea pigs with HSV-1, strain C42, on their backs has been described earlier, as well as the scoring system used to determine the therapeutic effect of treatment [1, 2, 13].

SYNTHESIS OF NUCLEOSIDE ANALOGUES

[E]-5-(1-Propenyl)-2'-deoxyuridine

This compound was synthesized according to Ruth and Bergstrom [23].

5-(1-Propenyl)-1-β-D-arabinofuranosyluracil

The method of synthesis was analogous to the one described by Ruth and Bergstrom [23] for [E]-5-(1-propenyl)-2'-deoxyuridine. 1-β-D-Arabinofuranosyluracil (1.0 g) suspended in 4 ml of H_2O was added to $Hg(OAc)_2$ (1.44 g) in 15 ml of H_2O and the solution was stirred at 50–60°C for 5 h. NaOAc (0.81 g) was added. After 12 h the suspension was cooled to room temperature, and 3.5 ml of allylchloride and 4.5 ml of 0.1 M Li_2PdCl_4 in MeOH were added followed by $CuCl_2 \cdot H_2O$ (0.42 g). After 6 h the suspension was treated with H_2S, filtered and chromatographed in $CHCl_3$–MeOH (4 : 1) on a silica gel (250 g) column, and 5-allyl-1-β-D-arabinofuranosyluracil (430 mg, 37%) was recovered. To a solution of 5-allyl-1-β-D-arabinofuranosyluracil (0.22 g) in 10 ml of 95% EtOH $Rh(Ph_3P)_3Cl$ (50 mg) was added during stirring and the mixture was refluxed. After 22 h the λ_{max} had shifted from 268 to 295 nm. The mixture was then extracted six times with 5 ml portions of 10% EtOH. The extract was concentrated and chromatographed on Biogel P-2 (2 × 30 cm) in 10% EtOH giving 5-(1-propenyl)-1-β-D-arabinofuranosyluracil (73 mg, 33%). UV (H_2O): λ_{max} 295 nm (ϵ = 8200), 237 nm (ϵ = 12,650), λ_{min} 267 nm (ϵ = 4640). MS: *m/e*, 284 (M^+). NMR (D_2O): δ = 1.77 (d, CH_3, J = 4.9 Hz), 3.87 (m, H-5'), 3.96 (m, H-4'), 4.13 (t, H-3', J = 5.4 Hz), 4.41 (t, H-2', J = 5.4 Hz), 6.15–6.23 (m, CH = CH, H-1'), 7.88 (s, C_6-H).

RESULTS

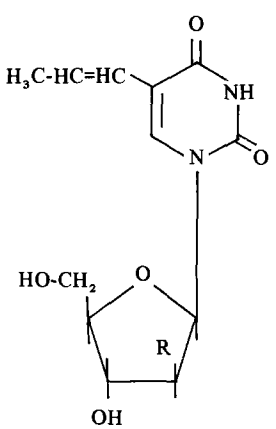
Anti-herpes activity in cell culture

The ability of [E]-5-(1-propenyl)-2′-deoxyuridine, and 5-(1-propenyl)-1-β-D-arabinofuranosyluracil (Fig. 1) to reduce plaque formation by HSV-1 and HSV-2 strains in cell culture is shown in Table 1. [E] -5-(1-Propenyl)-2′-deoxyuridine was an effective inhibitor of HSV-1 but not of HSV-2 multiplication. A variation in sensitivity between different HSV-1 strains was observed, and a thymidine kinase-negative (TK^-) strain as well as an acycloguanosine-resistant strain (ACG^r) of HSV-1 were resistant to [E]-5-(1-propenyl)-2′-deoxyuridine. On the other hand, a phosphonoformate-resistant strain (PFA^r) of HSV-1 was possibly more sensitive than its parent strain. The change of the sugar moiety in 5-(1-propenyl)-2′-deoxyuridine to arabinose led to an almost total loss of inhibition. However, 5-(1-propenyl)-1-β-D-arabinofuranosyluracil caused a slight reduction in plaque formation of the PFA^r HSV-1 strain (Table I). A comparison of the effect on HSV-1 C42 by [E]-5-(1-propenyl)-2′-deoxyuridine, 5-(1-propenyl)-1-β-D-arabinofuranosyluracil and their bromovinyl analogues showed a higher anti-herpes activity for the bromovinyl compounds (Table 2).

The HSV-1 plaque reduction caused by [E]-5-(1-propenyl)-2′-deoxyuridine could be reversed by thymidine as shown in Table 3. Deoxyuridine was less effective than thymidine in reducing the plaque inhibition.

Inhibition of thymidine phosphorylation

Cell-free assays with thymidine kinase from HSV-1, HSV-2 and uninfected cells were



R = H [E] -5-(1-Propenyl)-2′-deoxyuridine
R = OH 5-(1-Propenyl)-1-β-D-arabinofuranosyluracil

Fig. 1. Structure of [E]-5-(1-propenyl)-2′-deoxyuridine and 5-(1-propenyl)-1-β-D-arabinofuranosyluracil.

TABLE 1

Inhibition of HSV plaque formation by [E]-5-(1-propenyl)-2′-deoxyuridine and 5-(1-propenyl)-1-β-D-arabinofuranosyluracil

Virus strain		% Inhibition of plaque formation				
		[E]-5-(1-Propenyl)-2′-deoxyuridine			5-(1-Propenyl)-1-β-D-arabinofuranosyluracil	
		1 μM	10 μM	100 μM	100 μM	250 μM
HSV-1	C42	53	91	95	0	12
HSV-1	F2	39	68	–	–	–
HSV-1	KJ502	33	92	–	–	–
HSV-1	C42 PFA^r	91	99	–	50	65
HSV-1	C42 ACG^r	–	9	–	–	–
HSV-1	TK$^-$	–	0	–	-18	8
HSV-2	91075	–	–	45	–	0
HSV-2	72	–	–	30	–	0
HSV-2	B4327 UR2	–	–	-33	–	10

TABLE 2

Inhibition of HSV-1 strain C42 plaque formation

Compound	% Inhibition			
	0.1 μM	1 μM	10 μM	100 μM
[E]-5-(1-Propenyl)-2′-deoxyuridine	17	53	91	95
[E]-5-(2-Bromovinyl)-2′-deoxyuridine	> 99	–	–	–
5-(1-Propenyl)-1-β-D-arabinofuranosyl-uracil	–	–	–	0
5-(2-Bromovinyl)-1-β-D-arabinofuranosyl-uracil	–	80	> 90	–

TABLE 3

Reversion of plaque inhibition by [E]-5-(1-propenyl)-2′-deoxyuridine

Compound	% Inhibition of HSV-1 C42 plaque formation
10 μM [E]-5-(1-propenyl)-2′-deoxyuridine	93
10 μM thymidine	12
100 μM deoxyuridine	-8
10 μM [E]-5-(1-propenyl)-2′-deoxyuridine + 10 μM thymidine	-14
10 μM [E]-5-(1-propenyl)-2′-deoxyuridine + 10 μM deoxyuridine	60
10 μM [E]-5-(1-propenyl)-2′-deoxyuridine + 100 μM deoxyuridine	22

used to determine the ability of the nucleoside analogues to prevent phosphorylation of ^{3}H-labelled thymidine. As shown in Table 4, unlabelled thymidine, but not the nucleoside analogues, reduced the phosphorylation of ^{3}H-labelled thymidine by Vero cell thymidine kinase. [E] -5-(1-Propenyl)-2′-deoxyuridine, 5-(2-bromovinyl)-1-β-D-arabinofuranosyluracil and [E]-5-(2-bromovinyl)-2′-deoxyuridine effectively reduced the phosphorylation by HSV-1 thymidine kinase. 5-(1-Propenyl)-1-β-D-arabinofuranosyluracil did not interfere to the same extent. Of the tested nucleoside analogues only [E]-5-(1-propenyl)-2′-deoxyuridine and [E]-5-(2-bromovinyl))-2′-deoxyuridine showed a slight effect on the phosphorylation of thymidine by HSV-2 thymidine kinase.

Effect of [E]-5-(1-propenyl)-2′-deoxyuridine on cellular and viral DNA synthesis

As shown in Fig. 2, [E]-5-(1-propenyl)-2′-deoxyuridine preferentially reduced HSV-1 DNA synthesis in infected Vero cells. When viral DNA synthesis was inhibited by 63% at 100 μM concentration, a 39% inhibition of cellular DNA synthesis was observed. In uninfected Vero cells less than 50% inhibition of cellular DNA synthesis was seen at 300 μM of [E]-5-(1-propenyl)-2′-deoxyuridine (data not shown).

Effect of [E]-5-(1-propenyl)-2′-deoxyuridine on cutaneous HSV-1 infection in guinea pigs

The therapeutic effect of topically applied [E]-5-(1-propenyl)-2′-deoxyuridine

TABLE 4

Effect of nucleosides on thymidine phosphorylation by cellular and viral thymidine kinase

Compound	Concentration (μM)	% Inhibition of thymidine phosphorylation		
		Vero TK	HSV-1 TK	HSV-2 TK
Thymidine	5	59	66	49
	10	87	79	60
	100	80	97	87
[E]-5-(1-Propenyl)-2′-deoxyuridine	5	–	60	-3
	10	–	68	5
	100	10	100	53
5-(1-Propenyl)-1-β-D-arabinofuranosyluracil	5	–	21	-16
	10	–	40	-11
	100	26	87	-8
[E]-5-(2-Bromovinyl)-2′-deoxyuridine	5	–	79	1
	10	–	87	17
	100	14	100	63
5-(2-Bromovinyl)-1-β-D-arabinofuranosyluracil	5	–	61	-6
	10	–	72	-3
	100	19	84	14

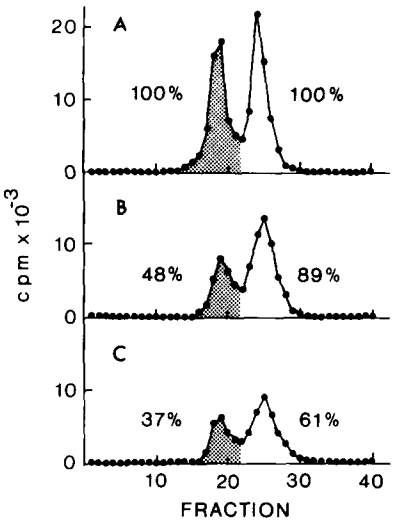


Fig. 2. Inhibition of HSV-1 and Vero cell DNA synthesis in infected cells by [E]-5-(1-propenyl)-2′-deoxyuridine. Viral and cellular DNA were labelled with ortho[^{32}P]phosphate and separated as described in Methods. The shaded area denotes viral DNA. A) Control. B) 10 μM [E]-5-(1-Propenyl)-2′-deoxyuridine. C) 100 μM [E]-5-(1-Propenyl)-2′-deoxyuridine. The DNA synthesis is given as percentage remaining activity. [^{3}H] Thymidine-labelled DNA from infected and untreated cells was used as an internal density marker (not shown). HSV-1 strain C42 was used.

on HSV-1 strain C42-infected guinea pig skin was determined and the result is shown in Fig. 3. A reduction in the cumulative lesion score from 21.0 ± 1.0 (S.D.) to 6.8 ± 2.8 (S.D.) was noticed when the compound was used at a 1% concentration in dimethylsulfoxide (DMSO) and compared to the effect of placebo (DMSO).

Cellular toxicity of [E]-5-(1-propenyl)-2′-deoxyuridine

The influence of [E]-5-(1-propenyl)-2′-deoxyuridine on cell growth was determined in Vero cells. A 50% inhibition of cell growth after 48 h of treatment was observed at a concentration of 100 μM. An increase by 50% in cell volume occurred at this concentration (data not shown).

DISCUSSION

The nature of the 5-substituent has been correlated to anti-herpes activity for several pyrimidine analogues [5, 6, 9, 24]. Some compounds, containing a 2-halogenated vinyl group in the 5-position, especially [E]-5-(2-bromovinyl)-2′-deoxyuridine, have shown a high anti-herpes activity in cell culture and a low cellular toxicity [7]. The introduction of a methyl group instead of bromine in [E]-5-(2-bromovinyl)-2′-deoxyuridine resulted in a lower anti-herpetic activity but the same difference in inhibition of type 1 and type 2 strains was observed (Table 1). This corresponds to the results reported

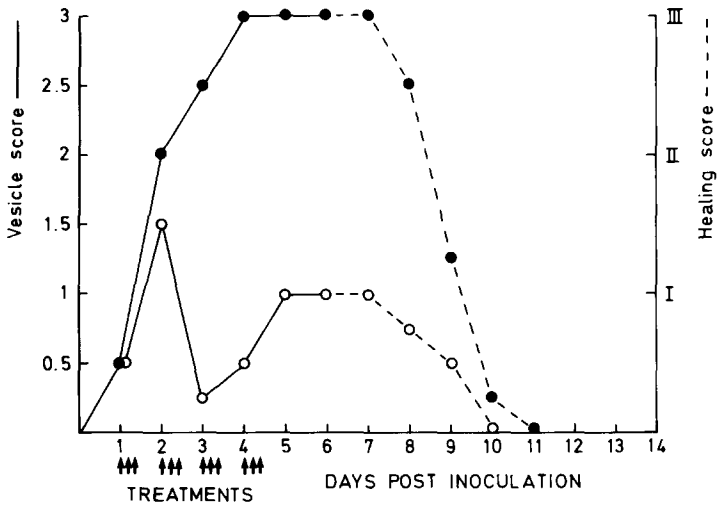


Fig. 3. Effect of [E]-5-(1-propenyl)-2′-deoxyuridine on cutaneous HSV-1 infection in guinea pigs. A topical treatment with 20 μl of a 1% solution in dimethylsulfoxide was initiated 24 h post inoculation. Three daily treatments were given during 4 days. The results are averages from four treated skin areas. ●—●, Skin areas treated with dimethylsulfoxide; ○- - -○, skin areas treated with 1% [E]-5-(1-propenyl)-2′-deoxyuridine.

by De Clercq [5] for [E]-5-(1-propenyl)-2′-deoxyuridine. Similar results have also recently been reported by Cheng et al. [3], who found a 2-log reduction in the virus titre of HSV-1 at 5 μM and of HSV-2 at 23 μM of [E]-5-(1-propenyl)-2′-deoxyuridine. A preferential inhibition of HSV-1 as compared to HSV-2 seems to be common to several 5-substituted pyrimidine nucleosides [5, 6, 8, 9, 24].

When arabinose was exchanged for deoxyribose in 5-(1-propenyl)-2′-deoxyuridine, a decreased antiviral activity was observed (Table 1). A decreased activity was also observed when arabinose was exchanged for deoxyribose in [E]-5-(2-bromovinyl)-2′-deoxyuridine (Table 2). A change from deoxyribose to arabinose has earlier been reported not to change the anti-herpetic activity of 5-ethyl-2′-deoxyuridine but to decrease the cellular toxicity [6]. With propyl as a 5-substituent the change to arabinose seems to lead to a decrease in antiviral activity [6, 21], in parallel to the present results. The low anti-herpetic activity of 5-(1-propenyl)-1-β-D-arabinofuranosyluracil in cell culture (Table 1) corresponds to an earlier report where a 50% inhibition of cytopathic effect was found at 35 μM (10 μg/ml) [20] for one HSV-1 strain and at a 10 times higher concentration for an HSV-2 strain. A bromovinyl 5-substituent instead of a 1-propenyl group gave a more inhibitory compound for both the deoxyribose and the arabinose derivatives (Table 2).

The lack of effect on thymidine kinase-negative and acycloguanosine-resistant HSV-1 strains showed the necessity of a viral thymidine kinase for the antiviral effect of [E]-

5-(1-propenyl)-2′-deoxyuridine (Table 1). It was also evident that the inhibition could be reversed by addition of thymidine to the cell culture (Table 3), further indicating the requirement of a phosphorylation by thymidine kinase. Deoxyuridine was less effective in reversing the inhibition by [E]-5-(1-propenyl)-2′-deoxyuridine, suggesting that thymidylate synthetase is not the target enzyme for this compound [5]. Cheng et al. [3] have reported that thymidine in more than 10-fold molar excess did not reverse the inhibition of HSV-1 and a 4-fold excess only partly reversed the inhibition of HSV-2 by [E]-5-(1-propenyl)-2′-deoxyuridine. It is not clear whether the results differ due to the use of different HSV strains or to the fact that Cheng et al. [3] used a yield reduction assay after infecting with a multiplicity of 5–10, while the results in Table 3 are plaque reduction determinations.

It was found that [E]-5-(1-propenyl)-2′-deoxyuridine could inhibit the phosphorylation of thymidine in a cell-free kinase assay using HSV-1 thymidine kinase (Table 4). This inhibition was similar to that of [E]-5-(2-bromovinyl)-2′-deoxyuridine. These results, together with the cell culture data, indicate that [E]-5-(1-propenyl)-2′-deoxyuridine has to be phosphorylated by HSV-1 thymidine kinase to exert an anti-herpes activity. The arabinose nucleoside also seemed to be phosphorylated by HSV-1 thymidine kinase (Table 4), indicating that its lack of anti-herpes activity could be due to a lack of further phosphorylation to triphosphate, or that the triphosphate is not inhibitory to HSV-1 DNA polymerase. The latter possibility seems more likely, since HSV-1 C42 PFA^r, which has an altered DNA polymerase [11], was more sensitive to the arabinose nucleoside than HSV-1 C42 and the altered polymerase could have a stronger affinity for a triphosphate. The lack of effect on HSV-2 multiplication could possibly be explained by an inability of HSV-2 thymidine kinase to phosphorylate these nucleoside analogues. The phosphorylation results for [E]-5-(1-propenyl)-2′-deoxyuridine corresponds to the K_m values determined by Cheng et al. [3] using cellular and viral thymidine kinases.

When the effect of [E]-5-(1-propenyl)-2′-deoxyuridine on HSV-1 and cellular DNA synthesis was studied in infected cells, a weak selectivity was seen (Fig. 2), indicating that a phosphorylated derivative (probably the triphosphate) of [E]-5-(1-propenyl)-2′-deoxyuridine is somewhat more inhibitory to HSV-1 DNA polymerase than to the cellular DNA polymerase(s). This pattern of inhibition is similar to that earlier observed for [E]-5-(2-bromovinyl)-2′-deoxyuridine [12, 18].

Very few nucleoside analogues have been therapeutically active when applied topically to HSV-1-infected guinea pig skin [12, 14]. However, a significant therapeutic effect was observed when a 1% solution of [E]-5-(1-propenyl)-2′-deoxyuridine was used. This corresponds to a similar effect seen for [E]-5-(2-bromovinyl)-2′-deoxyuridine in the guinea pig model (unpublished observations).

A low cellular toxicity was observed for [E]-5-(1-propenyl)-2′-deoxyuridine when both cellular DNA synthesis and cell proliferation were measured. The results agree with those of Cheng et al. [3]. The increase in cell volume at inhibitory concentrations indicates that the cytotoxic effect could be due to an inhibition of DNA synthesis.

This study has shown that a selective anti-herpes compound, dependent on HSV-1

thymidine kinase and selective for HSV DNA synthesis, can be obtained when 1-propenyl is used as a 5-substituent in deoxyuridine, and that a change to arabinose decreases the antiviral activity.

ACKNOWLEDGEMENTS

The excellent help with cell cultures and animal experiments by M. Berg, G. Brännström and K. Stenberg and the typing of the manuscript by M. Kropp and G. Norlin are gratefully acknowledged. Dr. L. Kenne at the University of Stockholm kindly assisted with NMR determinations.

REFERENCES

1 Alenius, S., Dinter, Z. and Öberg, B. (1978) Therapeutic effect of trisodium phosphonoformate on cutaneous herpesvirus infection in guinea pigs. Antimicrob. Agents Chemother. 14, 408–413.
2 Alenius, S. and Öberg, B. (1978) Comparison of the therapeutic effects of five antiviral agents on cutaneous herpesvirus infection in guinea pigs. Arch. Virol. 58, 227–288.
3 Cheng, Y.-C., Grill, S., Ruth, J. and Bergstrom, D.E. (1980) Anti-herpes simplex virus and anti-human cell growth activity of E-5-propenyl-2′-deoxyuridine and the concept of selective protection in antivirus chemotherapy. Antimicrob. Agents Chemother. 18, 957–961.
4 Cheng, Y.-C. and Ostrander, M. (1976) Deoxythymidine kinase induced in HeLa TK$^-$ cells by herpes simplex virus type 1 and type 2. J. Biol. Chem. 251, 2605–2610.
5 De Clercq, E. (1980) Antiviral and antitumor activities of 5-substituted 2′-deoxyuridines. Methods and Findings Exp. Clin. Pharmacol. 2, 253–267.
6 De Clercq, E., Descamps, J., Barr, P.J., Jones, A.S., Serafinowski, P., Walker, R.T., Huang, G.F., Torrence, P.F., Schmidt, C.L., Mertes, M.P. Kulikowski, T. and Shugar, D. (1979) Comparative study of the potency and selectivity of antiherpes compounds. In: Antimetabolites in Biochemistry, Biology and Medicine, eds. J. Skoda and P. Langen (Pergamon Press, Oxford and New York) pp. 275–285.
7 De Clercq, E., Descamps, J., De Somer, P., Barr, P.J., Jones, A.S. and Walker, R.T. (1979) (E)-5-(2-Bromovinyl)-2′-deoxyuridine; a potent and selective anti-herpes agent. Proc. Natl. Acad. Sci. U.S.A. 76, 2947–2951.
8 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.
9 De Clercq, E. and Torrence, P.F. (1978) Nucleoside analogs with selective antiviral activity. J. Carbohydrates, Nucleosides, Nucleotides, 5, 187–224.
10 Elion, G.B., Furman, P.A., Fyfe, J.A., De Miranda, P., Beauchamp, L. and Schaeffer, H.J. (1977) Selectivity of action of an antiherpic agent, 9-(2-hydroxyethoxymethyl)guanine. Proc. Natl. Acad. Sci. U.S.A. 74, 5716–5720.
11 Eriksson, B. and Öberg, B. (1979) Characteristics of herpesvirus mutants resistant to phosphonoformate and phosphonoacetate. Antimicrob. Agents Chemother. 15, 758–762.
12 Eriksson, B., Öberg, B. and Gauri, K.K. (1981) Inhibition of herpesvirus DNA polymerase by nucleotides derived from acyclonucleosides which do not suppress herpesvirus replication. In: Design of Inhibitors of Viral Functions, ed. K.K. Gauri (Academic Press, New York) (in press).
13 Helgstrand, E., Eriksson, B., Johansson, N.G., Lannerö, B., Larsson, A., Misiorny, A., Norén, J.O., Sjöberg, B., Stenberg, K., Stening, G., Stridh, S., Öberg, B., Alenius, S. and Phillipson, L. (1978) Trisodium phosphonoformate, a new antiviral compound. Science 201, 819–821.

14 Helgstrand, E., Flodh, H., Lernestedt, J.-O., Lundström, J. and Öberg, B. (1980) Trisodium phosphonoformate: antiviral activities, safety evaluations and preliminary clinical results. In: Developments in Antiviral Chemotherapy, eds. L.H. Collier and J. Oxford (Academic Press, New York) pp. 63–83.

15 Herrmann, E.C., Jr. (1961) Plaque inhibition test for detection of specific inhibitors of DNA containing viruses. Proc. Soc. Exp. Biol. Med. 107, 142–145.

16 Kulikowski, T., Zawadski, Z., Shugar, D., Descamps, J. and De Clercq, E. (1979) Synthesis and antiviral activities of arabinofuranosyl-5-ethyl-pyrimidine nucleosides. Selective antiherpes activity of 1-(β-D-arabinofuranosyl)-5-ethyluracil. J. Med. Chem. 22, 647–653.

17 Larsson, A. and Öberg, B. (1981) Selective inhibition of herpesvirus DNA synthesis by foscarnet. Antiviral Res. 1, 55–62.

18 Larsson, A. and Öberg, B. (1981) Selective inhibition of herpesvirus DNA synthesis by acycloguanosine, 2′-fluoro-5-iodo-aracytosine and (E)-5-(2-bromovinyl)-2′-deoxyuridine. Antimicrob. Agents Chemother. 19, 927–929.

19 Lee, L.-S. and Cheng, Y.-C. (1976) Human deoxythymidine kinase. J. Biol. Chem. 251, 2600–2604.

20 Machida, H., Kuninaka, A., Yoshino, H., Ikeda, K. and Mizuno, Y. (1980) Antiherpes viral activity and inhibitory action on cell growth of 5-alkenyl derivatives of 1-β-D-arabinofuranosyluracil. Antimicrob. Agents Chemother. 17, 1030–1031.

21 Machida, H., Sakata, S., Kuninaka, A., Yoshino, H., Nakayama, C. and Saneyoshi, M. (1979) In vitro antiherpesviral activity of 5-alkyl derivatives of 1-β-D-arabinofuranosyluracil. Antimicrob. Agents Chemother. 16, 158–163.

22 Prusoff, W.H. (1959) Synthesis and biological activities of iododeoxyuridine, an analog of thymidine. Biochim. Biophys. Acta 32, 295–296.

23 Ruth, J.L. and Bergstrom, D.E. (1978) C-5 substituted pyrimidine nucleosides. I. Synthesis of C-5 allyl, propyl and propenyl uracil and cytosine nucleosides via organopalladium intermediates. J. Org. Chem. 43, 2870–2876.

24 Shannon, W.M. and Schabel, F.M., Jr. (1980) Antiviral agents as adjuncts in cancer chemotherapy. Pharmacol. Ther. 11, 263–390.

25 Stenberg, K. (1981) Cellular toxicity of pyrophosphate analogues. Biochem. Pharmacol. 30, 1005–1008.

26 Watanabe, K.A., Reichman, U., Hirota, K., Lopez, C. and Fox, J.J. (1979) Nucleosides. 110. Synthesis and antiherpes virus activity of some 2′-fluoro-2′-deoxyarabinofuranosylpyrimidine nucleosides. J. Med. Chem. 22, 21–24.