Acta virol. **28** : 282—286, 1984

DIFFERENTIAL ANTIHERPES ACTIVITY OF THE (*E*)- AND (*Z*)-ISOMERS OF 5-(2-FLUOROVINYL)-2′-DEOXYURIDINE (FVUdR[1])

J. REEFSCHLÄGER, *D. BÄRWOLFF, *P. LANGEN

Chair of Virology, Medical Department, Humboldt University Berlin, DDR — 1040 Berlin, G.D.R.,
and *Department of Cell Kinetics, Central Institute of Molecular Biology, G.D.R. Academy of Sciences, DDR — 1115 Berlin-Buch, G.D.R.

Received December 5, 1983

Summary. — Four novel analogues of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine [(*E*)-BrVUdR] — the (*E*)- and (*Z*)-isomer of 5-(2-fluorovinyl)-UdR (FVUdR), (*Z*)-5-(2-carboxy-2-fluorovinyl)-UdR [(*Z*)-COOH-FVUdR], and (*E*)-5-(2-ethoxyvinyl)-UdR [(*E*)--EOVUdR] were compared with the reference compounds (*E*)--BrVUdR and 5-vinyl-UdR (VUdR) for their inhibitory effects on plaque formation of herpes simplex virus type 1 (HSV-1 strain 77) and type 2 (HSV-2 strain 82) in human embryonic lung fibroblast (HELF) cell cultures. (*Z*)-FVUdR and (*Z*)-COOH--FVUdR were completely inactive against HSV-1 and HSV-2 ($ID_{50} > 500$ μM). For the other analogues the following order of decreasing potency was found: (*E*)-BrVUdR > VUdR > (*E*)-FVUdR >> (*E*)-EOVUdR (against HSV-1) and VUdR > > (*E*)-BrVUdR > (*E*)-FVUdR >> (*E*)-EOVUdR (against HSV--2).

Key words: herpes simplex virus; antiviral activity; (E)-5-(2-fluorovinyl)-2′-deoxyuridine; (Z)-5-(2-fluorovinyl)-2′-deoxyuridine; (E)--5-(2-ethoxyvinyl)-2′-deoxyuridine

Introduction

Among the great number of synthesized 5-substituted-2′-deoxyuridine (UdR) derivatives, those with a 5-vinyl structure are the most potent and selective inhibitors of herpes simplex virus type 1 (HSV-1) replication (De Clercq, 1980). Substitution of a halogene (chlorine, bromine, iodine) for an H atom on the C-2 of the vinyl group yields compounds, e.g. (*E*)-5-(2-bromovinyl)-UdR [(*E*)-BrVUdR], with an enhanced anti-HSV-1 potency and selectivity (De Clerq *et al.*, 1979; Reefschläger *et al.*, 1982*a*). The (*E*)-5-(2-halogenovinyl) substituent also confers excellent anti-HSV-1 properties

[1]) Presented in part at the conference on "Synthetic Oligonucleotides in Molecular Biology," August 15—20, 1982, Uppsala, Sweden

to 2′-deoxycytidines (De Clerq *et al.*, 1982*a*) and to 1-β-D-arabinofuranosyluracil (araU) nucleosides (Machida *et al.*, 1981; Busson *et al.*, 1981; De Clerq *et al.*, 1982*b*; Reefschläger *et al.*, 1983). The replication of herpes simplex virus type 2 (HSV-2), however, inhibited only at 100—1000 fold higher concentrations of these analogues (De Clerq *et al.*, 1980, 1982*a*; Busson *et al.*, 1981; Machida *et al.*, 1981: Reefschläger *et al.*, 1982*b*, 1983). Therefore, recent efforts have been directed to the elucidation of the general structure (type of base, sugar and substituents) as well as the specific sterical and electronical configuration of the 5-(2-X-vinyl) substituent of pyrimidine nucleosides required for maximal anti-HSV-1 and anti-HSV-2 activity. The replacement of the deoxyribose in (*E*)-BrVUdR analogues by various substituted and nonsubstituted sugars (Busson *et al.*, 1981; De Clerq *et al.*, 1982*b*) as well as the replacement of the halogen in (*E*)-5-(2-halogenovinyl)-UdR analogues by substituents with different steric and electronic properties (De Clerq *et al.*, 1981; Reefschläger *et al.*, 1984) have not lead to more potent or selective compounds.

When the deoxyribose of (*E*)-5-(1-propenyl)-UdR (Cheng *et al.*, 1980; De Clerq *et al.*, 1981), a strongly antiherpetic (*E*)-5-(2-X-vinyl)-UdR derivative (X = CH_3), is replaced by arabinose, a virtually inactive analogue results (Stening *et al.*, 1981). The reduced antiherpes activity of the (*Z*)-isomer of BrVUdR (Jones *et al.*, 1981; Reefschläger *et al.*, 1984) and of the 5-(2,2--dibromovinyl)-UdR (Br_2VUdR) (Reefschläger *et al.*, 1984) emphasize the importance of the C-2 H atom of the bromovinyl group in cis (*Z*) position.

We have now synthesized the (*E*)- and the (*Z*)-isomers of 5-(2-fluorovinyl) -UdR (FVUdR) (Bärwolff *et al.*, 1981), the ether derivative (*E*)-5-(2-ethoxyvinyl)-UdR [(*E*)-EOVUdR] and the disubstituted (*Z*)-5-(2-carboxy-2-fluorovinyl)-UdR [(*Z*)-COOH-FVUdR] and compared their effects against HSV-1 and HSV-2 with those of (*E*)-BrVUdR and 5-vinyl-UdR (VUdR).

Materials and Methods

The antiviral potency of the analogues was evaluated by a plaque inhibition assay in HELF cell cultures. The cells were grown in Eagle's minimal essential medium (MEM; Institut für Immunpräparate und Nährmedien, Berlin, G.D.R.) containing Earle salts supplemented with 10% calf serum (5% foetal and 5% neonatal; Gibco Bio-Cult Laboratory, Glasgow, Scotland (MEM/10). Neomycin (Spofa, Prague, Czechoslovakia) was used as an antibiotic. Twenty ml--scintillation vials (diameter 2.5 cm) were used for the estimation of the effective concentration range of the compounds, whereas precise determinations of the 50% inhibition (ID_{50}) values were performed in plaque assays with 50 ml-tissue culture flasks. Nearly confluent monolayers (24 hr after seeding) were infected with 0.1 or 0.2 ml of a virus suspension, respectively, yielding about 50 or 100 plaques per bottle. The origin, differentiation and biological characteristics of the recent clinical isolates HSV-1 (strain 77) and HSV-2 (strain 82) (obtained by courtesy of P. Wutzler, Medizinische Akademie, Erfurt, G.D.R.) were described previously (Reefschläger *et al.*, 1982*b*). After a 1 hr adsorption at 37 °C a methocel (0.5% w/v; Methylcellulose MC U.S.P. 4000 cP, Fluka AG, Buchs SG, Switzerland) overlay medium (MEM/10) without antibiotics and appropriate substance dilutions (1/20 of the total overlay volume) were added to each culture. Plaques were counted after direct addition of a neutral red solution to the overlay at 48 hr p.i. with HSV-2 (strain 82) and 72 hr p.i. with HSV-1 (strain 77). The resulting plaque counts were expressed as percentages of the counts obtained for untreated infected control cultures and were plotted on a decimal scale against the logarithm of the concentration. The ID_{50} values were determined graphically from the dose-response curves. The individual data listed in Table 1 represent average

Table 1. Inhibition of HSV-1 and HSV-2 plaque formation in HELF cell cultures by some 5-substituted 2-deouxyuridine analogues

5-Substituent	ID_{50} (μM)*		Ratio ID_{50} (HSV-2) / ID_{50} (HSV-1)
	HSV-1 (strain 77)	HSV-2 (strain 82)	
(*E*)—CH=CH—Br	0.036	5.5	153
—CH=CH_2	0.32	2.2	7
(*E*)—CH=CH—F	3.8	9.2	2
(*E*)—CH=CH—OC_2H_5	90	1000	11
(*E*)—CH=CF—COOH	> 500	> 500	1
(*Z*)—CH=CH—F	> 500	> 500	1

* Inhibitory dose $_{50}$ = concentration required to reduce the number of plaques by 50% compared with an untreated infected control culture

values for two to three separate experiments with three concentrations each within the inhibition range of the compounds and triplicate cultures.

The reference compounds (*E*)-5-(2-bromovinyl)-and 5-vinyl-UdR were prepared as described previously (Langen and Bärwolff, 1975; Reefschlä ger *et al.*, 1982*a*). The synthesis and preparation of (*E*)- and (*Z*)-FVUdR as well as (*E*)-EOVUdR and (*Z*)-COOH-FVUdR will be described elsewhere (D. Bärwolff *et al.*, in preparation).

Results and Discussion

We found marked differences between the antiviral activities of the various 5-(2-X-vinyl)-UdR analogues (Table 1). The activity decreased in the following order towards HSV-1 (strain 77) and HSV-2 (strain 82), respectively: (*E*)-BrVUdR > VUdR > (*E*)-FVUdR >> (*E*)-EOVUdR and VUdR > (*E*) -BrVUdR > (*E*)-FVUdR >> (*E*)-EOVUdR. The (*Z*)-isomer of FVUdR and the disubstituted derivative (*Z*)-COOH-FVUdR were devoid of activity against either HSV type up to the concentration of 500 μM. The results clearly showed that, while the (*E*)-bromo substitution of one of the vinyl C-2 H atoms led to a 10-fold higher anti-HSV-1 activity, the fluoro substitution lowered the activity of the parent VUdR compound 10-times. Thus (*E*)-FVUdR had a 100-times lower anti-HSV-1 effect than (*E*)-BrVUdR. On the other hand, the anti-HSV-2 potential of the parent VUdR compound was decreased by both the bromo and the fluoro substitution (Table 1). The (*E*)-5-(2-halogenovinyl)-UdR analogues (with the halogen = chloro, bromo, iodo) varied little in their antiherpetic activity (De Clercq *et al.*, 1981). Whereas (*Z*)-BrVUdR was 20—100 times less active against HCV-1 than (*E*)-BrVUdR (Jones *et al.*, 1981; Reefchläger *et al.*, 1984), (*Z*)-FVUdR was completely inactive against HSV-1 and HSV-2 (Table 1). The fact that (*E*)-5-(2-carboxyvinyl)-UdR had a slight effect on HSV-1 (strain 77) (ID_{50} = = 300 μM) (Reefschläger *et al.*, 1984) which was completely abolished by an additional fluoro substitution [(*Z*)-COOH-FVUdR], attested to the importance of the H atom in (*Z*)-position, confirming our results with 5-(2,2-dibromovinyl)-UdR (Reefschläger *et al.*, 1984) (Fig. 1).

The low antiherpetic activity of [(*E*)-5-(2-ethoxyvinyl]-UdR carrying a new type of 5-substituent, was in agreement with our previous conclusion (Reefschläger *et al.*, 1982*a*) that 2′-deoxypyrimidine nucleosides with more than three atoms in the C-5 side chain possessed only weak antiherpetic activity. It remains to be seen whether another ether derivative, (*E*)-5-

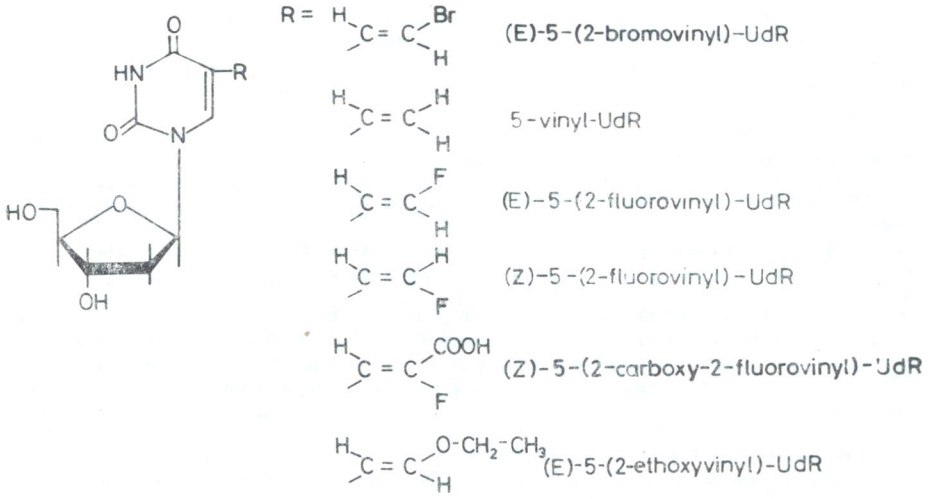

Fig. 1.
Structural formulae of 5-substituted 2′-deoxyuridine (UdR) analogues

(2-methoxyvinyl)-UdR, has any activity. The ratios of the ID_{50} values for HSV-2 and HSV-1 (column 4, Table 1) show that (*E*)-FVUdR and the parent VUdR compound suppressed both HSV types to similar degrees in contrast to (*E*)-BrVUdR which discriminated 100-fold between HSV-1 and HSV-2.

The main conclusions of this investigation could be summarized as follows: *first*, a fluorine in the (*Z*)-position of the C-2 of the 5-vinyl substituent leads to a loss of antiherpetic activity: *second*, replacement of the bromine in (*E*)-BrVUdR by fluorine results in a severe reduction of anti-HSV-1 activity and: *third*, HSV-1 and HCV-2 differ only slightly in their sensitivity to (*E*)-FVUdR. Although our both present and previous data indicate the importance of the (*Z*)-position of the vinyl-C-2 hydrogen, the study of (*E*)-5-(2-bromo-2-fluorovinyl)-UdR and (*Z*)-5-(2-bromo-2-fluorovinyl)-UdR may be useful for establishment of further relationship between the structure and activity of these compounds.

Acknowledgements. We wish thank Kathleen Schröder for excellent technical assistance and Dr. Cornelia Schroeder for help in preparing the manuscript. We would like to thank Professor H. A. Rosenthal for his support and criticism.

References

Bärwolff, D., Reefschläger, J., and Langen, P. (1981): Synthesis of 5-(2-fluorovinyl)-2′-deoxyuridine. *Nucleic Acids Res. Symp. Ser.* **9**, 45—47.

Busson, R., Colla, L., Vanderhaeghe, H., and De Clercq, E. (1981): Synthesis and antiviral activity of some sugar-modified derivatives of (E)-5-(2-bromovinyl)-2′-deoxyuridine. *Nucleic Acids Res. Symp. Ser.* **9**, 49—52.

Cheng, Y.-C., Grill, S., Ruth, J., and Bergstrom, D. E. (1980): Antiherpes 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.

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. natn. Acad. Sci. U.S.A.* **76**, 2947—2951.

De Clercq, E. (1980): Antivital and antitumor activities of 5-substituted 2′-deoxyuridines. *Meth. Find. exptl. clin. Pharmacol.* **2**, 253—267.

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

De Clerq, E., Verhelst, G., Descamps, J., and Bergstrom, D. E. (1981): Differential inhibition of herpes simplex viruses, Type 1 (HSV-1) and type 2 (HSV-2), by (*E*)-5-(2-X-vinyl)-2′-deoxyuridines. *Acta microbiol. Acad. Sci. hung.* **28**, 307—312.

De Clerq, E., Balzarini, J., Descamps, J., Huang, G. -F., Torrence, P. F., Bergstrom, D. E., Jones, A. S., Serafinowski, P., Verhelst, G., and Walker, R. T. (1982 *a*): Antiviral, antimetabolic and cytotoxic activities of 5 substituted 2′ deoxycytidines. *Mol. Pharmacol.* **21**, 217—223.

De Clerqc, E., Busson, R., Colla, L., Descamps, J., Balzarini, J., and Vanderhaeghe, H. (1982*b*): Antiviral activities of sugar modified derivatives of (*E*)-5-(2-bromovinyl)2′-deoxyuridine, pp. 1065—1067. In P. Periti and G. G. Grassi (Eds): *Current Chemotherapy and Immunotherapy.* American Society for Microbiology, Washington DC.

Jones, A. S., Rahim, S. G., Walker, R. T., and De Clercq, E. (1981): Synthesis and antiviral properties of (*Z*)-5-(2-bromovinyl)-2′-deoxyudridine. *J. Med. Chem.* **24**, 759—760.

Langen, P., and Bärwolff, D. (1975): On the mode of action of 5-vinyl-2′-deoxyuridine. *Biochem. Pharmacol.* **24**, 1907—1910.

Machida, H., Sakata, S., Kunikaka, A., and Yoshino, H. (1981): Antiherpesviral and anticellular effects of 1-β-D-arabinofuranosyl-*E*-5-(2-halogenovinyl)uracils. *Antimicrob. Agents Chemother.* **20**, 47—52.

Reefschläger, J., Bärwolff, D., Engelmann. P., Langen, P., and Rosenthal, H. A. (1982): Efficiency and selectivity of (*E*)-5-(2-bromovinyl)-2′-deoxyuridine and some other 5-substituted 2′-deoxypyrimidine nucleosides as antiherpes agents. *Antiviral Res.* **2**, 41—52.

Reefschläger, J., Wutzler, P., Thiel, K. -D., Bärwolff, D., Langen, P., Sprössig, M., and Rosenthal, H. A. (1982 b): Efficacy of (*E*)-5-(2-bromovinyl-)2′-deoxyuridine against herpes simplex virus strains in cell culture and agaist experimental herpes encephalitis in mice. *Antiviral Res.* **2**, 255—265.

Reefschläger, J., Herrmann, G., Bärwolff, D., Schwarz, B., Cech, D., and Langen, P. (1983): Antiherpesviral potential 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. *Antiviral Res*, **3**, 175—187.

Reefschläger, J., Bärwolff, D., Herrmann, G., and Langen, P. (1984): Antiherpes activity of some novel analogues of (*E*)-5-(2-bromovinyl)-2′-deoxyruidine [(*E*)-BrVUdR] in two different cell lines. *Acta virol.* **28**, 1—10.

Stening, G., Gotthammar, B., Larsson, A., Alenius, S., Johansson, N. G., and Oberg, B. (1981): Antiherpes activity of (*E*)-5-(1-propenyl)-2′-deoxyuridine and 5-(1-propenyl)-1-β-D-arabinofuranosyluracil. *Antiviral Res.* **1**, 213—223.