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Antiviral activity of uridine 5'-diphosphate glucose analogues against some enveloped viruses in cell culture

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Summary

Twenty five analogues of uridine 5'-diphosphate glucose were screened against herpes simplex type 2, vaccinia virus, Sindbis virus and African swine fever virus. After screening, the compound 5'-[[[[(2'',3'',4'',6''-tetra-*O*-benzoyl-α-D-glucopyranosyl)oxi]carbonyl]amino]sulfonyl]uridine (**2**), the synthesis of which has been reported (Camarasa et al., J. Med. Chem. 28, 40–46, 1985), was selected for further study.

This compound showed in vitro activity against all viruses tested. The replication of herpes virus type 2 and African swine fever virus was completely inhibited at 100 µg/ml and 150 µg/ml respectively; vaccinia virus and Sindbis virus were inhibited to a lesser extent. The compound may inhibit several steps in the viral replication process.

Uridine diphosphatehexose analogues; Uridine analogues; Herpes simplex 2 virus; Vaccinia virus, Sindbis virus; African swine fever virus

Introduction

Potent and selective antiherpes drugs are known (Came and Caliguiri, 1982; Carrasco and Smith, 1984; De Clercq and Walker, 1984). Their selectivity is based on the recognition of these compounds by herpesvirus-specified enzymes, such as

thymidine kinase (TK), which are different from those present in the uninfected cell. For example, 9-(2-hydroxyethoxymethyl)-guanine [Acyclovir] (Fyfe et al., 1978), (E)-5-(2-bromovinyl)-2'-deoxyuridine [BVdU] (Cheng et al., 1981) or 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-iodocytosine [FIAC] (Lopez et al., 1980) are selectively phosphorylated by the herpesvirus-induced TK and preferentially inhibit viral DNA polymerase. These modified nucleoside derivatives can also be incorporated into DNA, which might possibly lead to long-term side effects. Therefore, new selective antiviral agents acting through different mechanisms of action may be desirable. Some particular processes that occur in the virus-infected cell, such as glycosylation of viral proteins necessary for the assembly of several enveloped viruses, may be considered as suitable targets for selective antiviral drugs (Klenk and Schwarz, 1982; Rapp and Wigdahl, 1984).

A new type of uridine 5'-diphosphate glucose (UDP-Glc, **1**, Fig. 1) analogue, having antiviral activity against herpes simplex type 1 (HSV-1), has recently been reported (Camarasa et al., 1985). The structure of this new family of antivirals has been chemically modified (Camarasa et al., 1986; Fernández-Resa et al., 1986; Fiandor et al., 1987) and structure-activity relationships have been established with regard to their anti-HSV-1 effects. These compounds were initially designed to interfere with the glycosylation process and, in fact, inhibit the glycosylation of HSV-1 proteins (Camarasa et al., 1985). However, their mode of action is more complex since they also inhibit DNA synthesis (unpublished data).

In this paper we report on the antiviral activity of the above uridine 5'-diphosphate glucose analogues against enveloped DNA viruses, namely, Herpes simplex virus type 2 (HSV-2), vaccinia virus (VV) and African swine fever virus (ASFV). Sindbis virus, as a member of the enveloped RNA viruses, was also studied.

Materials and Methods

Cell culture

Vero cells were grown in Dulbecco's modified Eagle's medium with glutamine,
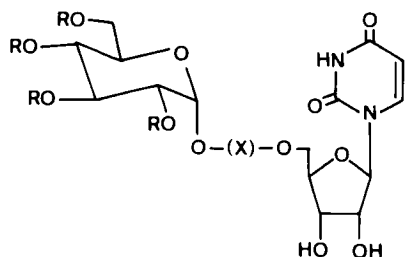
1. R = H ; X = PO$_2$H −O−PO$_2$H

2. R = C$_6$H$_5$CO ; X = CO−NH−SO$_2$

Fig. 1. Chemical structure of compounds 1 and 2.

10% calf serum, and either 0.85% sodium bicarbonate for flask cultures, or 3.7% sodium bicarbonate for cultures in 24- or 96-well plates (Costar, Cambridge, MA, U.S.A.), incubated at 37°C in a 5% CO_2 atmosphere with 95% humidity. The maintenance medium was supplemented with 2% calf serum. Vero cells, media and sera were supplied by Flow Labs., Scotland, U.K.

Viruses

HSV-2 Lovelace strain was obtained from the Centro Nacional de Microbiología, Virología e Immunología Sanitarias, Madrid, Spain, by the courtesy of Dr. R. Nájera; ASFV adapted to growth in Vero cells was kindly supplied by Dr. E. Viñuela (Enjuanes et al., 1976), Centro de Biología Molecular, Madrid, Spain; VV was obtained from vaccinia germ-free calf lymph by filtration and maintained in our laboratory by serial passage in Vero and chick embryo cells; SV was kindly supplied by Istituto Superiore di Sanitá, Roma, Italy. Virus stocks were prepared in Vero cell culture, divided into 0.5 ml samples, frozen, and stored at −70°C until used, and their titres were determined by plaque assay.

Drugs

According to their chemical structure the UDP-Glc analogues were divided into three groups: sugar-modified (Table 1), nucleoside-modified (Table 2) and diphosphate-modified (Table 3). They were assayed and the antiviral activity determined. Chemical structures and references for their synthesis are given in Tables 1, 2 and 3.

Chemicals and isotopes

L-[^{35}S]-methionine (1075 Ci/mmol) was purchased from Radio-Chemical Centre, Amersham, U.K.; DMSO, Merck, Darmstadt, F.R.G.; sodium dodecylsulfate (SDS), Serva, Heidelberg, F.R.G.; trichloroacetic (TCA), Merck, Darmstadt, F.R.G.; and Ready-Sol™ HP, Beckman Instruments, Madrid, Spain; ribavirin, ICN Nutritional Biochemicals, Cleveland, OH, U.S.A.; acyclovir (ACV) was kindly supplied by Burroughs Wellcome, Research Triangle Park, NC, U.S.A.; and 5-iodo-2'-deoxyuridine (IDU), Sigma, St. Louis, MO, U.S.A.

Cellular toxicity

The effects of the drugs on cell proliferation were measured as follows. Vero cells suspended in growth medium containing test compound at various concentrations were seeded into 24-well plates with 5×10^4 cells/ml in each well. Four wells were used for each sample. After 72 h, monolayers were trypsinized and the number of viable cells was determined by the trypan blue exclusion method. Cultures without compound were included as a control, and MTC_{50} (minimum toxic concentration required to inhibit 50% cell replication) values were calculated three days after seeding the cells in the presence or absence of the compounds.

Effect of drugs on protein synthesis

The effect of different concentrations of each drug were determined in mono-

layers of Vero cells growing in 96-well plates (two wells were used for each drug concentration). Forty eight hours after treatment at 37°C, the medium was removed and methionine-free maintenance medium, containing [^{35}S]methionine (10 µCi/ml), was added and incubated for 1 h. The medium was then removed, the cells washed with PBS solution and precipitated with TCA 5% during 5 min. The cell monolayers were washed with ethanol, dried and dissolved in 250 µl 0.1 N NaOH plus 1% SDS. Samples of 50 µl were examined for radioactivity in an Intertechnique scintillation spectrometer. The counts obtained from two wells were compared with controls without drug, and IP$_{50}$ (concentration required to inhibit 50% of protein synthesis) values were calculated.

Effect of drug concentration on virus yield

Vero cells were grown to 100% confluency in 24-well plates. Cells were infected with the viruses at a multiplicity of infection (MOI) of 0.5. After 60 min of adsorption for HSV-2, VV and SV and 90 min for ASFV at 37°C, the inoculum was removed, the monolayers washed with PBS, and maintenance medium with various concentrations of drug was added (using three wells per sample).

A control without drug was used for each virus. When the virus control induced 100% cytopathic effect (CPE), usually after 48 h for HSV-2, VV, and SV, or 72 h for ASFV, the medium and cells were removed with a rubber policeman, three wells being used for each sample, and the cells were disrupted by sonication. Cell debris was removed by centrifugation and the supernatants were frozen at −70°C until titration by plaque assay. For the plaque assay method, monolayers of Vero cells growing in 24-well plates were infected with three different dilutions of each sample (three wells for each dilution) and after the adsorption period indicated above for the different viruses, the unadsorbed virus was removed, the monolayers washed with PBS and overlaid with double concentrated Dulbecco's modification of Eagle's medium with 3.7% sodium bicarbonate and 0.9% noble agar at equal amount. After 72 h for HSV-2, VV and SV, and 7 days for ASFV, the solid overlay was removed, the cultures were stained with 1% crystal violet in ethanol and the plaques were counted. The antiviral potency of the drugs was determined by estimating the values of drug concentration required to reduce virus yield by 50% of the virus control (MIC$_{50}$). All assays were repeated at least three times.

Virucidal effect

Viruses at high concentration were mixed with the drug (compound 2) at the maximal dose at which no cytotoxicity was shown. The mixtures were placed at 37°C with periodical shaking. Samples were removed at 0,2,4,6 h, diluted to concentrations in which the drug was not active and titrated in Vero cells by plaque assay.

Effect of drug addition time on the inhibition of viral replication

Confluent Vero cell monolayers in 24-well plates were infected at a MOI of 1 for HSV-2, VV, SV and 0.5 for ASFV. After virus adsorption (60 min for HSV-2, VV and SV, and 90 min for ASFV) considered as zero time, compound 2 (100

TABLE 1   Antiviral activity of sugar modified analogues of uridine-5'-diphosphate glucose.

Sugar—O—C—N—S—O (structure with uracil base and ribose ring, RO OR)
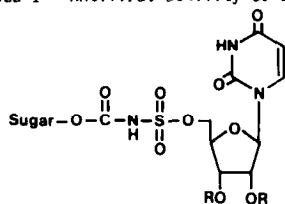
Compd	Sugar[Ref][a]	R	HSV-2	VV	SV	ASFV	MTC$_{50}$ µg/ml
				MIC$_{50}$µg/ml(TI)[b]			
2	2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl[2]	H	26(7.4)	25(7.7)	55(3.5)	18(10.7)	192
3	2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl[2]	(CH$_3$)$_2$C	30(3.3)	20(5)	>100	0.1(1000)	100
4	2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl[2]	(CH$_3$)$_2$C	10(10)	1(100)	>100	40(2.3)	100
5	2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl[2]	(CH$_3$)$_2$C	>100	>100	>100	>100	>100
6	2,3,4-6-tetra-O-palmitoyl-α-D-glucopyranosyl[2]	(CH$_3$)$_2$C	>100	>100	>100	25(2)	50
7	2-acetamido-3,4,6-tri-O-benzoyl-2-deoxy-α-D-gluco-pyranosyl[9]	(CH$_3$)$_2$C	>100	100	>100	>100	100
8	2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-gluco-pyranosyl[2]	(CH$_3$)$_2$C	100	>100	>100	100	100
9	2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl[9]	(CH$_3$)$_2$C	>100	>100	40(2.5)	100	100
10	2,3,4,6-tetra-O-benzoyl-α-D-mannopyranosyl[9]	H	>200	200	>200	25(8)	200
11	2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl[9]	(CH$_3$)$_2$C	>100	>100	>100	100	100
12	2,3,4,6-tetra-O-acetyl-β-D-mannopyranosyl[9]	(CH$_3$)$_2$C	>100	>100	100	>100	>100
13	2,3,4,6-tetra-O-benzoyl-α-D-galactopyranosyl[9]	(CH$_3$)$_2$C	35(4.2)	46(3.2)	100	25(6)	150
14	2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl[9]	(CH$_3$)$_2$C	50(4.4)	150	100(2.8)	NT[c]	220
15	3,4,6-tri-O-benzoyl-2-deoxy-α-D-arabino-hexo-pyranosyl[9]	(CH$_3$)$_2$C	66(3.7)	75(3.3)	100	235	250
16	3,4,6-tri-O-benzoyl-2-deoxy-α-D-arabino-hexo-pyranosyl[9]	H	300	300	200	>100(3)	300
17	3,4,6-tri-O-benzoyl-2-deoxy-β-D-arabino-hexopyranosyl[9]	(CH$_3$)$_2$C	50(2)	50(2)	>100	50(2)	100
18	3,6-di-O-benzoyl-2,4-dideoxy-α-D-threo-hexopyranosyl[9]	(CH$_3$)$_2$C	200	>200	>200	50(4)	200

a) Reference for the synthesis.

b) TI, Therapeutic index = MTC$_{50}$/MIC$_{50}$

c) NT = Not tested.

μg/ml) was added at different times (2,3,6,8,10,20 and 22 h). After 24 h of incubation for HSV-2, VV and SV, and 72 h for ASFV, the cells and medium were removed, disrupted, centrifuged and titrated.
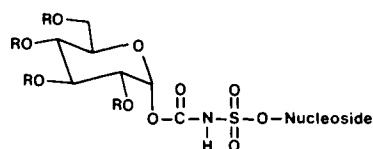
Results

Twenty five analogues of uridine 5'-diphosphate glucose were screened against HSV-2, VV, SV and ASFV. Their structures as well as the MIC_{50} and MTC_{50} values are shown in Tables 1, 2 and 3. Compounds showing a therapeutic index (TI = MTC_{50}/MIC_{50})$\geqslant$5 were considered as selective antiviral agents.

Tetra-O-benzoyl and tetra-O-benzyl derivatives of α-D-glucopyranose (**2, 3, 4**) showed TI values against HSV-2 and VV in the range of 5–100. Analogues of UDP-hexose having other protecting groups of the glucose hydroxyls, such as acetyl (**5**) or palmitoyl (**6**) or having other hexoses such as mannose (**9, 10, 11, 12**), glucosamine (**7, 8**), galactose (**13, 14**), 2-deoxyglucose (**15, 16** and **17**) and 2,4-dideoxyglucose (**18**) did not show significant activity (Table 1). Structural modifications of the nucleoside moiety (Table 2) or "diphosphate" bridge (Table 3) led to decreased activity.

None of the compounds tested showed significant activity against Sindbis virus.

Analogues of UDP-hexose having 2,3,4,6-tetra-O-benzoylated residues of glucose (**2, 3, 19, 20, 22, 24** and **25**), mannose (**10**) and galactose (**13**) showed the

TABLE 2 Antiviral activity of nucleoside-modified analogues of uridine-5'-diphosphate glucose.
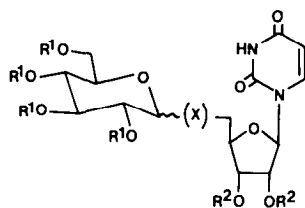

Comp	Nucleoside [Ref][a]	R	MIC_{50} μg/ml (TI)[b]				MTC_{50} μg/ml
			HSV-2	VV	SV	ASFV	
19	3'-O-acetylthymidin-5'-yl[3]	C_6H_5CO	25(6)	>200	200	10(15)	150
20	3'-O-acetylthymidin-5'-yl[3]	$C_6H_5CH_2$	75	75	50	10(7.5)	75
21	uracil-1-yl-methoxyethyl[3]	$C_6H_5CH_2$	25(3)	75	25(3)	25(3)	75
22	3'-O-acetyl-2'-deoxyuridin-5'-yl[3]	C_6H_5CO	NT[c]	NT[c]	NT[c]	50(6)	300

a) Reference for the synthesis.

b) TI, therapeutic index = MTC_{50}/MIC_{50}

c) NT = Not tested.

TABLE 3 Antiviral activity of diphosphate-modified analogues of uridine-5'-diphosphate glucose.

Compd	X Ref [a]	R^1	R^2	HSV-2	MIC$_{50}$ µg/ml (TI)[b] VV	SV	ASFV	MTC$_{50}$ µg/ml
23	NH-CO-NH-SO$_2$-O (α-anomer)[8]	C$_6$H$_5$CH$_2$	(CH$_3$)$_2$	NT[c]	200	75(2.6)	NT[c]	200
24	NH-CO-NH-SO$_2$-O (β-anomer)[8]	C$_6$H$_5$CO	(CH$_3$)$_2$C	50(6)	50(6)	200	20(15)	300
25	CH$_2$-NH-Co-NH-SO$_2$-O (α-anomer)[8]	C$_6$H$_5$CH$_2$	(CH$_3$)$_2$C	45(2.2)	150	150	10(10)	100
26	O-(CH$_2$)$_3$-[d]	C$_6$H$_5$CO	(CH$_3$)$_2$C	75	100	100	25(3)	75

a) Reference for the synthesis.

b) TI, Therapeutic index = MTC$_{50}$/MIC$_{50}$

c) NT = not tested.

d) This bridge links the anomeric position of glucose and N-3 of the uridine, not 0-5' as indicated
in the general formula. Thus, compound 25 is 3-N-[[(2",3",4",6"-tetra-O-benzoyl-α-D-glucopyranosyl)
oxy]propyl]-2'.3'-isopropylideneuridine.

TABLE 4 Antiviral activity of compound 2 in infected Vero cell cultures.

Antiviral activity		Therapeutic index	
Virus	MIC$_{50}$ (µg/ml)	(I) MTC$_{50}$/MIC$_{50}$	(II) IP$_{50}$/MIC$_{50}$
HSV-2	26 (0.1)[a]	7.4 (3.000)[a]	9.1
VV	25 (25)[b]	7.7 (8.0)[b]	9.5
ASFV	18 (25)[c]	10.6 (8.0)[c]	13.2
SV	55	3.5	4.3

MIC$_{50}$ = Concentration required to reduce virus yield by 50%.

MTC$_{50}$ = Concentration required to inhibit cell multiplication by 50%:
192 µg/ml (compound 2), 300 µg/ml (acyclovir), 200 µg/ml (IDU and ribavirin).

IP$_{50}$ = Concentration required to inhibit protein synthesis by 50% (237 µg/ml).

In parentheses, the data for (a) acyclovir, (b) IDU, (c) ribavirin.

greatest activity against ASFV, with a TI value of 1000 for (3). The presence of other protecting groups of the hexose hydroxyls, such as benzyl (4), acetyl (5, 8, 11, 12), acetamido (7, 8) and palmitoyl (6), or the absence of one or two benzoyl groups on the glucose moiety, such as in 15–18, led to a loss of antiviral activity. Other structural modifications of the nucleoside (Table 2) or bridge (Table 3) also afforded compounds with moderate anti-ASFV activity.

Compound 2 was selected for further study because it was active against three viruses (HSV-2, VV and ASFV), and the only one showing some activity against SV.

The concentrations at which compound 2 showed antiviral activity (expressed as MIC_{50}) were well below the concentrations required to produce cell cytotoxicity (TC) and inhibition of protein synthesis (IP) (expressed as MTC_{50} (192 μg/ml) and IP_{50} (237 μg/ml) respectively (Table 4)). Based on these two cytotoxicity parameters, two therapeutic indexes were calculated: Index I = MTC_{50}/MIC_{50} and index II = IP_{50}/MIC_{50}. In all cases index II was greater than index I, which reflects the sensitivity of the two methods for detecting cellular and metabolic cytotoxicity, respectively.

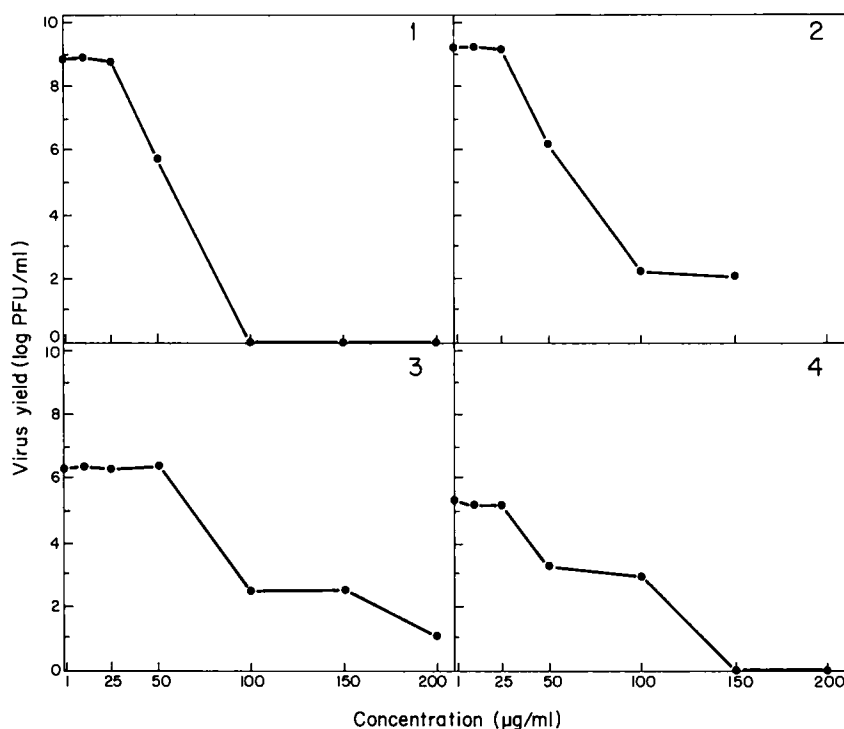Table 4 also shows the MIC_{50} and MTC_{50} values, obtained under the same ex-

Fig. 2. Virus dose-response curves of compound 2 against HSV-2 (1), VV (2), SV (3) and ASFV (4). Monolayers of Vero cells were infected as indicated in Materials and Methods and virus yields were determined by plaque assay at 48 h after infection for HSV-2, VV and SV, and at 72 h for ASFV.

perimental conditions, for three well-known antiviral agents, acyclovir, 5-iodo-2'-deoxyuridine, and ribavirin. Compound 2 was less active than acyclovir against HSV-2. It showed a potency similar to that of IDU against VV and was slightly more active than ribavirin against ASFV.

The effect of compound 2 on virus replication is shown in Fig. 2. At a concentration below 25 μg/ml no reduction in HSV-2, VV or ASFV yield was observed. For SV no reduction in virus yield was observed at concentrations up to 50 μg/ml. At a concentration of 100 μg/ml compound 2 strongly inhibited viral replication. The production of new infectious HSV-2 and ASFV progeny was completely inhibited at a concentration of 100 μg/ml and 150 μg/ml, respectively. The inhibitory effect of compound 2 did not show a linear concentration dependence and disappeared at a concentration below 25 μg/ml.

The effect of different times of addition of compound 2 on viral replication is shown in Fig. 3. The most pronounced inhibitory effect was observed when the compound was added within the first four hours after virus adsorption. When added 4–10 h after infection, compound 2 still achieved an inhibitory effect on viral replication, but when it was added later than 10 h after virus infection, no antiviral activity was observed.
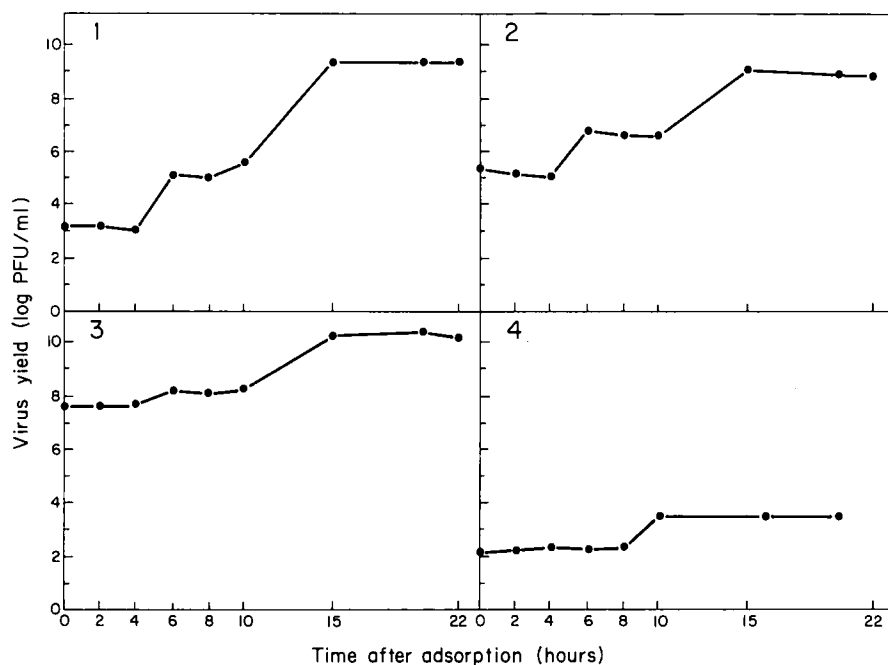

Fig. 3. Effect of addition time of compound 2 on yield of HSV-2 (1), VV (2), SV (3) or ASFV (4). Vero cell monolayers were infected at 1 PFU/cell (HSV-2, VV, SV) or 0.5 PFU/cell (ASFV). After virus adsorption, compound 2 (100 μg/ml) was added at the times indicated on the x-axis. Virus yield was determined at 24 h (at 72 h for ASFV).

TABLE 5   Virucidal activity of compound 2 against HSV-2, VV, SV and ASFV.

Hours of contact[a]	HSV-2		VV		SV		ASFV	
	Untreated	Treated	Untreated	Treated	Untreated	Treated	Untreated	Treated
0	6.3×10^6	6.0×10^6	1.3×10^6	1.3×10^6	2.0×10^6	1.8×10^6	4.9×10^5	3.8×10^5
2	3.8×10^6	1.0×10^2	1.2×10^6	1.7×10^5	1.4×10^6	1.2×10^5	5.1×10^5	1.7×10^5
4	1.5×10^6	0.5×10^2	9.2×10^5	1.5×10^5	9.3×10^5	7.0×10^4	3.7×10^5	8.0×10^4
6	4.0×10^5	1.0×10^1	7.1×10^5	9.0×10^4	6.8×10^5	4.0×10^4	3.6×10^5	9.0×10^4

a) Virus or virus plus drug were incubated at 37°C during 6 hours with occasional shaking.
Every two hours a sample of each virus with or without drug was harvested and titrated
on Vero cell monolayers.

Table 5 shows the direct virucidal effect of compound 2 on infectious virus particles. There was a dramatic virucidal effect on HSV-2, a one log loss of titer for VV and SV, while ASFV was not significantly affected.

Discussion

Several of the 25 UDP-hexose analogues tested showed activity against HSV-2, VV, ASFV, and, to a lower extent, SV. The antiviral action against HSV-1 has been reported previously (Camarasa et al., 1985, 1986; Fernández-Resa et al., 1986; Fiandor et al., 1987).

The favorable effect of the benzyl or benzoyl protecting groups of the glucose hydroxyls on the antiviral activity of UDP-hexose analogues 2-25 seems to be related, in the case of HSV-2 and VV, to an adequate partition coefficient. The presence of other protecting groups with low (acetyl or acetamido) or high (palmitoyl) lipophilic character results in compounds devoid of antiviral activity. The antiviral effects on these two viruses are also dependent on the structure and stereochemistry of the hexopyranosyl residue, since UDP-hexose derivatives with hexose moieties (glucosamine, mannose, galactose, 2-deoxy-glucose, 2,4-dideoxy-glucose) different from glucose did not show significant activity.

The activity against ASFV was not influenced by the stereochemistry of the sugar but seemed to depend mainly on the presence of a tetrabenzoyl protected hexopyranose moiety (glucose, mannose, galactose). This indicates that, in addition to good transport properties of the molecule, other factors, such as ease of hydrolysis of the protecting groups, are important for the observed anti-ASFV activity.

From the two-step curves obtained for the virus yields upon addition of compound 2 at different times after viral infection one might infer that the compound inhibits both an early and late step, or at least more than one step, in the virus replicate cycle. Several nucleoside analogues having antiviral activity, such as Ara-A and ribavirin, are also able to block viral replication at more than one step (Carrasco and Vázquez, 1984). The complex mode of action of the compounds may be rationalized in function of their structure. On the one hand, they are analogues of UDP-glucose and this may explain their inhibitory effect on protein glycosyla-

tion (Camarasa et al., 1985; Fiandor et al., 1987). On the other hand, compound 2 and its possible metabolites resulting from hydrolysis of the "diphosphate" bridge may be considered as 5'-O-sulfamoyluridine derivatives, thus nucleotide analogues, and this might be responsible for their inhibitory effects on DNA synthesis (unpublished data) as well as protein glycosylation. Nucleocidin (Morton et al., 1969), ascamycin (Osada and Isono, 1985) and dealanylascamycin (Osada and Isono, 1985) are naturally occurring nucleoside antibiotic derivatives which owe their biological activity to their nucleotide-like structure, and this may also be the case for compound 2. Several nucleotides have been reported to inhibit protein glycosylation. For example, CMP inhibits transfer of NANA from CMP-NANA to desialated fetuin by sialyltransferase (Bernacki, 1975) and the 5'-monophosphate of BVdU also inhibits protein glycosylation (Olofsson et al., 1985). Thus, compound 2 might interfere with viral DNA synthesis when present shortly after viral adsorption and may also inhibit protein glycosylation when added late after infection.

In addition to the above mentioned inhibitory mechanism we found that compound 2 had a marked virucidal effect on HSV-2. This virucidal effect, corresponding to a decrease in viral infectivity of 4 logs for HSV-2, was not observed with the other viruses tested. Whether compound 2 would be clinically effective against herpes simplex infections remains to be seen. Additional in vivo animal studies are required to assess the therapeutic potentials of compound 2.

Acknowledgements

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