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Selective inhibitory effects of (*S*)-9-(3-hydroxy-2-phosphonyl-methoxypropyl) adenine and 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouracil on seal herpesvirus (phocid herpesvirus 1) infection in vitro

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Summary

From a selection of 25 antiviral compounds with specific anti-herpes activity or broad-spectrum antiviral properties, two compounds, namely (*S*)-9-(3-hydroxy-2-phosphonyl-methoxypropyl)adenine and 1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouracil, appeared particularly effective in inhibiting the cytopathogenicity of seal herpesvirus (phocid herpesvirus 1).

Phocid herpesvirus 1; Seal herpesvirus; Antiviral compound

Introduction

Seal herpesvirus (SeHV) or phocid herpesvirus 1 is a highly pathogenic herpesvirus that has been recently isolated from the harbor seal (*Phoca vitulina*) during a lethal disease outbreak with symptoms of acute pneumonia and focal hepatitis in a specialized seal orphanage in The Netherlands [20]. The severity of the disease (about 50% mortality rate) and the isolation of SeHV as the causative agent of the disease have prompted the search for an effective antiviral chemotherapy. Therefore, various antiviral compounds were evaluated for their ability to inhibit SeHV infection in seal kidney (SeK) monolayer cell cultures. The compounds were

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selected on the basis of their known antiherpetic potential (i.e. against herpes simplex virus (HSV)) or broad-spectrum antiviral properties in general.

Materials and Methods

The compounds with the abbreviations used, their origin (source) and references to their synthesis and antiviral activity, are as follows:
5-iodo-2′-deoxyuridine (IDU): Ludeco (Brussels, Belgium) [10];
5-trifluoro-2′-deoxythymidine (TFT): Sigma Chemical Company (St. Louis, MO, U.S.A.) [10];
5-ethyl-2′-deoxyuridine (EDU): courtesy of E. Mauz and B. Hempel, Robugen GmbH (Esslingen/Neckar, F.R.G) [10];
5-(2-chloroethyl)-2-′-deoxyuridine (CEDU): courtesy of B. Rosenwirth, Sandoz Forschungsinstitut (Vienna, Austria) [6,13];
(*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU): Rega Institute for Medical Research, University of Leuven (Leuven, Belgium) [7,10];
carbocyclic BVDU (C-BVDU): Rega Institute for Medical Research [8,14];
5-iodo-2′-deoxycytidine (IDC): Serva Feinbiochemica (Heidelberg, F.R.G) [10];
1-β-D-arabinofuranosylcytosine (Ara-C): The Upjohn Company (Puurs, Belgium) [10];
9-β-D-arabinofuranosyladenine (Ara-A): Parke Davis and Company (Ann Arbor, MI, U.S.A.) [10];
1-β-D-arabinofuranosylthymine (Ara-T): courtesy of H. Machida, Yamasa Shoyu Company (Choshi, Japan) [10];
1-β-D-arabinofuranosyl-(*E*)-5-(2-bromovinyl)uracil (BVaraU): courtesy of H. Machida [7];
1-(2′deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodocytosine (FIAC): courtesy of J.J. Fox, Sloan-Kettering Institute (New York, NY, U.S.A.) [10,22];
1-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)-5-iodouracil (FIAU): courtesy of J.J. Fox [22];
9-(2-hydroxyethoxymethyl)guanine (Acyclovir, ACV): Burroughs Wellcome Company (Research Triangle Park, NC, U.S.A.) [10];
9-[(1,3-dihydroxy-2-propoxy)methyl]guanine (DHPG, also referred to as NDG, BIOLF 62 and BW 759U): courtesy of J.P.H. Verheyden, Syntex Research (Palo Alto, CA, U.S.A.) [12,16];
The (*R*)- and (*S*)-enantiomers of 9-(3,4-dihydroxybutyl)guanine [(*R*)- and (*S*)-DHBG]: courtesy of B. Öberg, Astra Läkemedel AB (Södertälje, Sweden) [11];
phosphonoformate trisodium salt hexahydrate (PFA): Sigma Chemical Company [19];
ribavirin: ICN Nutritional Biochemicals (Cleveland, OH, U.S.A.) [21];
(*S*)-9-(2,3-dihydroxypropyl)adenine [(*S*)-DHPA]: courtesy of A. Holý, Czechoslovak Academy of Sciences (Prague, Czechoslovakia) [9];
(*RS*)-3-adenin-9-yl-2-hydroxypropanoic acid [(*RS*)-AHPA] isobutyl ester: courtesy of A. Holý [4];

carbocyclic 3-deazaadenosine (C-c^{3}Ado): courtesy of J.A. Montgomery, Southern Research Institute (Birmingham, AL, U.S.A.) [5,18];
neplanocin A: courtesy of J. Murase, Toyo Jozo Company (Mifuku Ohito-Cho, Tagata-Gun, Shizuoka-Ken, Japan) [3];
9-(2-phosphonylmethoxyethyl)adenine (PMEA): courtesy of A. Holý [23].
(*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(*S*)-HPMPA]: courtesy of A. Holý [23].

Stock solutions of the test compounds were prepared at 1 mg/ml in tissue culture medium (F-10 maintenance medium, supplemented with 5% FCS, 100 IU/ml penicillin and 100 μg/ml streptomycin). Stock solutions of the test compounds were diluted in tissue culture medium at 300, 100, 30, 10 ... 0.01 μg/ml. Of each dilution 50 μl was mixed with 50 μl virus suspension, containing 100 $TCID_{50}$ SeHV, and the mixtures (100 μl) were then added to confluent SeK cell cultures in microtiter plates. Per well 50 μl tissue culture medium was added, and the cells were further incubated for 65 h at 37°C. Viral cytopathogenicity was then recorded, and antiviral activity was determined as the IC_{100}, or lowest concentration of the test compound required to inhibit viral cytopathogenicity by 100%. Control cell cultures were incubated with serial dilutions of the test compounds, without virus, to detect possible cytotoxicity due to the test compounds.

Cytotoxicity of the test compounds was monitored by measuring their inhibitory effect on the host cell DNA synthesis, based on a reduction in the incorporation of [*methyl*-^{3}H]dThd. Therefore, the test compounds were diluted at 1 or 10 times their IC_{100} in tissue culture medium. To each dilution (50 μl) 80 000 SeK cells (100 μl) and 0.5 μCi [*methyl*-^{3}H]dThd (10 μl) were added, and the mixtures were further incubated in microtiter plates for 24 h at 37°C. After freeze-thawing the amount of radioactivity incorporated into DNA was assessed, and the inhibition of DNA synthesis was expressed as a percentage relative to the control (not exposed to the test compound).

Results and Discussion

Of the 25 compounds that were evaluated, only two compounds, namely (*S*)-HPMPA and FIAU, proved markedly inhibitory to SeHV. These compounds completely inhibited the cytopathogenicity of SeHV for SeK cells at a concentration as low as 0.3 μg/ml. (*S*)-HPMPA and FIAU could be considered as truly selective inhibitors of SeHV, as they were themselves not cytotoxic (based on a microscopic evaluation of cell morphology) for the host cells at a concentration of 100 μg/ml; and, at a concentration 10 times higher than the concentration required to completely suppress the cytopathic effect of SeHV, (*S*)-HPMPA and FIAU had only minor effect on host cell DNA synthesis: 15% and 23% reduction in [*methyl*-^{3}H]dThd incorporation for (*S*)-HPMPA and FIAU, respectively (Table 1).

It is noteworthy that those compounds that have been previously recognized as selective inhibitors of HSV replication, i.e. EDU, CEDU, BVDU, C-BVDU, IDC, Ara-T, BVaraU, FIAC, ACV and DHBG [6,7,8,10,11,12,13,14,16,22] were to-

tally inactive against SeHV. Only those anti-herpes agents that show little, if any, specificity for HSV, i.e. IDU, TFT, Ara-C, Ara-A, DHPG and PFA, exhibited some activity against SeHV but only at a fairly high concentration (10 or 30 μl/ml). Moreover, IDU, Ara-C, Ara-A and PFA inhibited host cell DNA synthesis by about 50% at the concentration found effective against SeHV (Table 1), which indicates the lack of selectivity of their anti-SeHV activity.

With the broad-spectrum antiviral agents, ribavirin, (*S*)-DHPA, (*RS*)-AHPA, C-c^{3}Ado and neplanocin A, no marked anti-SeHV activity was noted (Table 1), which is perhaps not surprising, since these compounds are virtually inactive against herpesviruses at large [3,4,5,9,18].

The mechanism of the anti-SeHV action of (*S*)-HPMPA and FIAU remains to be determined. The anti-SeHV activity of (*S*)-HPMPA and FIAU may be related to their inhibitory activity against human cytomegalovirus (HCMV), since both (*S*)-HPMPA [23] and FIAU [2,15], are effective inhibitors of HCMV replication. But so is FIAC [2,15], and FIAC was found ineffective against SeHV (Table 1). It would

TABLE 1

Inhibitory effects of selected antiviral compounds against SeHV in cell cultures

Compound	Anti-SeHV activity	Cytotoxicity	
	IC_{100} (μg/ml)*	inhibition of DNA synthesis (%)	
		at 1 IC_{100}	at 10 IC_{100}
IDU	10	56	82
TFT	10	5	59
EDU	> 100	–	–
CEDU	> 100	–	–
BVDU	> 100	–	–
C-BVDU	> 100	–	–
IDC	> 100	–	–
Ara-C	30	71	95
Ara-A	30	62	95
Ara-T	> 100	–	–
BVaraU	> 100	–	–
FIAC	100	–	–
FIAU	0.3	6	23
ACV	> 100	–	–
DHPG	30	17	59
(*R*)-DHBG	> 100	–	–
(*S*)-DHBG	> 100	–	–
PFA	30	43	55
Ribavirin	> 100	–	–
(*S*)-DHPA	> 100	–	–
(*RS*)-AHPA	> 100	–	–
C-c^3 Ado	30	74	76
Neplanocin A	100	–	–
PMEA	30	29	74
(*S*)-HPMPA	0.3	13	15

* 100%-Inhibitory concentration, or concentration required to completely inhibit cytopathogenicity of 100 $TCID_{50}$ SeHV. Based on the incorporation of *methyl*-^{3}H dThd into DNA uninfected SeK cells.

seem interesting to examine how the other 2′-fluorinated compounds, i.e. FMAU, FEAU, FMAC [2,15], behave in their activity against SeHV.

Thus, based on the present data, (*S*)-HPMPA and FIAU would seem particularly promising candidates for the treatment of the SeHV infection in seals, for which no effective therapy currently exists. The ultimate usefulness of FIAU and (*S*)-HPMPA will depend on their therapeutic (toxicity/activity) ratio in vivo. The toxicity profile of FIAU is rather well established [17]; that of (*S*)-HPMPA is now under investigation. In vivo studies in seals will be carried out when a new outbreak of SeHV infection takes place in the seal orphanage in The Netherlands.

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