Selective Inhibitory Effect of (S)-9-(3-Hydroxy-2-Phosphonylmethoxypropyl)Adenine and 2'-Nor-Cyclic GMP on Adenovirus Replication In Vitro

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The inhibitory effects of 20 selected antiviral compounds on the replication of adenoviruses (types 1 to 8) in vitro were investigated. While 18 compounds were ineffective, 2 compounds, namely (S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine [(S)-HPMPA] and 9-[(2-hydroxy-1,3,2-dioxaphosphorinan-5-yl)oxymethyl]guanine P-oxide (2'-nor-cyclic GMP), were highly effective against all adenovirus types assayed in human embryonic fibroblast cultures. Their 50% inhibitory doses were 1.1 μg/ml for (S)-HPMPA and 4.1 μg/ml for 2'-nor-cyclic GMP. They were nontoxic for the host cells at the effective antiviral doses.

Adenoviruses (AVs) infect epithelial cells of mucous membranes, cornea, respiratory tract, intestinal tract, and other organs. The clinical manifestations caused by AV infections may vary considerably, but most of them are not severe and heal without special chemotherapy. In recent years, however, it has been reported that severe and occasionally fatal AV infections may occur in immunocompromised patients, i.e., patients with leukemia and recipients of kidney or bone marrow allografts (11, 14, 17). Also, epidemic keratoconjunctivitis, generally caused by AV type 8 or 19, is a frequently occurring and highly infectious disease. These reasons justify the search for antiviral compounds that are effective against AV replication. Of a series of 20 selected antiviral compounds that were evaluated in vitro, two congeners emerged as being particularly promising for the chemotherapy of AV infections.

Eight strains of adenovirus divided into four groups, i.e., types 3 and 7, group B; types 1, 2, 5, and 6 group C; type 8, group D and type 4, group E, were all isolated in our laboratory from patients with various AV infections and typed by the cross-neutralization test with standard type sera provided by the National Institute of Health, Tokyo, Japan. Viruses were passaged in human embryonic fibroblast (HEF) cell cultures three or four times, titrated, and stored at −80°C until use. HEF cell cultures were propagated in Eagle minimum essential medium supplemented with 10% heat-inactivated newborn calf serum–100 U of penicillin G per ml–50 μg of streptomycin per ml (growth medium). The HEF cells were checked for mycoplasma contamination and were found to be mycoplasma-free.

The origin of the compounds screened for anti-AV activity was as follows: 3'-bromo-3'-deoxyxyloA, 3'-iodo-3'-deoxyxyloA, 3'-chloro-3'-deoxyxyloA, 3'-azido-3'-deoxyAdo, 3'-azido-3'-deoxyaraA (xyloA, Ado, and araA are xyloadenosine, adenosine, and arabinofuranosyladenine, respectively; obtained from H. Wiedner and F. Eckstein, Max-Planck-Institut für Experimentelle Medizin, Göttingen, Federal Republic of Germany) (5); 3'-chloro-2',3'-dideoxy-BVUrd [BVUrd is (E)-5-(2-bromovinyl)uridine] (3); 3'-azido-2',3'-dideoxy-IxyloU (IxyloU is 5-iodoxylouridine); 3'-azido-2',3'-dideoxyxyloU (xyloU is xylouridine) (4); ribavirin

[1-(β-D-ribofuranosyl)-1,2,4-triazole-3-carboxamide; Virazole; ICN Nutritional Biochemicals, Cleveland, Ohio] (13); pyrazofurin [3-(β-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxamide; pyrazomycin; Calbiochem-Behring Corp., Lucerne, Switzerland] (9); 3-deazaguanine [6-amino-imidazo(4,5-c) pyridin-4(5H)-one; obtained from R. K. Robins, Nucleic Acid Research Institute, Costa Mesa, Calif.] (1); tubercidin [4-aminopyrrolo-(2,3-d)pyrimidine-β-D-ribofuranoside; Sigma Chemical Co., St. Louis, Mo.] (2); (S)-DHPA [(S)-9-(2,3-dihydroxypropyl)adenine; obtained from A. Holý, Czechoslovak Academy of Sciences, Prague, Czechoslovakia] (6); C-c³Ado (carbocyclic 3-deazaadenosine; obtained from J. A. Montgomery, Southern Research Institute, Birmingham, Ala.)(7);(S)-HPMPA{[(S)-9-(3-hydroxy-2-phosphonylmethoxypropyl)adenine (Fig. 1)]; obtained from A. Holý [E. De Clercq, A. Holý, I. Rosenberg, T. Sakuma, J. Balzarini, and P. C. Maudgal, Letter, Nature (London) 323:464–467, 1986]}; 2'-nor-cyclic GMP (cGMP) {9-[2-hydroxy-1,3,2-dioxaphosphorinan-5-yl)oxymethyl]guanine P-oxide (Fig. 1)} (16); ACV (acyclovir; Zovirax; Burroughs Wellcome Co., Research Triangle Park, N.C.); AraA (adenine arabinoside; Vira-A; Parke, Davis & Co., Ann Arbor, Mich.); BVDU [(E)-5-(2-bromovinyl)-2'-deoxyuridine] (8); PFA (phosphonoformate; Astra Läkemedel AB, Södertälje, Sweden) (10).

The antiviral assays were carried out in confluent HEF monolayers in 96-well, flat-bottom plates (diameter, 5 mm; Falcon; Becton Dickinson Labware, Oxnard, Calif.). The HEF monolayers were infected with 0.1 ml of virus suspension containing approximately 100 50% cell culture infective doses of AV. After adsorption of virus for 90 min at 37°C, to each well was then added 0.1 ml of maintenance medium (minimal essential medium, 2% newborn calf serum, and antibiotics at the concentrations indicated above) containing various concentrations of test compounds (250, 50, 10, 2, 0.4, and 0.08 μg/ml). Eight wells were used per compound concentration per virus strain. As the virus control, 0.1 ml of virus suspension and 0.1 ml of maintenance medium without compounds were added to the wells. The monolayers were incubated at 37°C for 7 days, and then the cytopathic effect (CPE) of AV was recorded microscopically. Although the rate of replication and the shape of CPE of AVs were different among the serotypes, the distinct CPE was ob-

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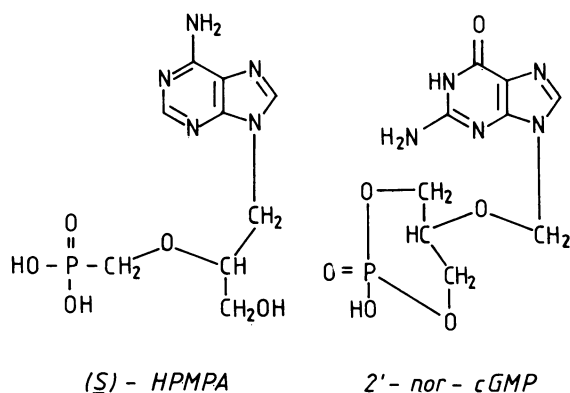
(S) - HPMPA   2'- nor - cGMP

FIG. 1. Structural formulas of (S)-HPMPA and 2'-nor-cGMP.

served after 7 days in the virus control of all serotypes. All assays were repeated twice with separate trays. The concentration of compound required to protect 50% of the wells completely against the viral CPE was determined as the 50% inhibitory dose (ID_{50}). The ID_{50} endpoints were calculated by the Reed-Muench method.

Cytotoxicity of the compounds for the host cells was examined by the inhibition of host cell DNA synthesis as described previously (12). Briefly, [methyl-^{3}H]thymidine was added at 0.25 μCi/10^5 HEF cells per well (diameter, 16 mm) together with various concentrations of test compounds. The amounts of [methyl-^{3}H]thymidine incorporated into acid-insoluble material were determined after 24 h of exponential growth of the cells. The ID_{50} for host DNA synthesis was defined as the concentration of compound required to reduce the [methyl-^{3}H]thymidine incorporation to 50% of the value obtained for the control cell cultures.

With the exception of (S)-HPMPA and 2'-nor-cGMP, none of the compounds was active against AV replication at the highest concentration tested (125 μg/ml). For 3'-azido-3'-deoxyado, pyrazofurin, and tubercidin, concentrations higher than 26, 32, and 0.4 μg/ml, respectively, could not be

tested because of cytotoxicity to the host cells (Table 1). Only two compounds, (S)-HPMPA and 2'-nor-cGMP, proved effective as inhibitors of AV replication at nontoxic concentrations (Table 1). They were effective against the eight types of AV. However, (S)-HPMPA was, at an average, 3 to 4 times more effective than 2'-nor-cGMP. Their mean ID_{50}s for the eight AV types were 1.1 and 4.1 μg/ml, respectively. They had no influence on host cell DNA synthesis at the effective antiviral concentrations. Based on the ID_{50} for AV replication (all types) and the ID_{50} for host cell DNA synthesis, the selectivity index of (S)-HPMPA was 47, as compared with 15 for 2'-nor-cGMP.

In recent years, several antiherpesvirus compounds have been described that can be administered both systemically and topically in the treatment of severe herpes simplex virus (HSV) and varicella-zoster virus (VZV) infections, e.g., ACV, araA, BVDU, PFA. Potential candidate antiviral drugs have also been reported for the treatment of some RNA virus infections, e.g., (S)-DHPA, C-c^{3}Ado, 3-deazaguanine. However, little, if any, progress has been made so far toward the development of an effective anti-AV agent. Antiherpesvirus compounds may be expected to be inactive against AV, because the herpesvirus-specified enzymes seem to be different from the enzymes involved in the replication of AV. It is not surprising, therefore, that the anti-herpes compounds ACV, araA, BVDU, and PFA used in this study were all ineffective as inhibitors of AV replication (Table 1).

Tolman et al. (16) reported that a cGMP analog, 2'-nor-cGMP, inhibits the replication of many DNA viruses, including AV type 2, cytomegalovirus and thymidine kinase-deficient HSV mutants. Recently, De Clercq et al. (Letter, Nature) introduced a new compound, (S)-HPMPA, as a highly potent and selective agent against a broad spectrum of DNA viruses, including HSV, VZV, cytomegalovirus, vaccinia virus, and thymidine kinase-deficient mutants of HSV and VZV. Apparently, the antiviral effects of 2'-nor-cGMP and (S)-HPMPA are not dependent on the help of a virus-specified thymidine kinase. Their exact mechanism of action remains to be elucidated. A putative target for their action

TABLE 1. Inhibitory effects of selected antiviral compounds on the replication of AV (types 1 to 8) in HEF cell cultures

Compound	ID_{50} (μg/ml) for the following								
	Group B, type 3	Group B, type 7	Group C, type 1	Group C, type 2	Group C, type 5	Group C, type 6	Group D, type 8	Group E, type 4	HEF
3'-Bromo-3'-deoxyxyloA	>125	>125	>125	>125	>125	>125	>125	>125	ND[a]
3'-Iodo-3'-deoxyxyloA	>125	>125	>125	>125	>125	>125	>125	>125	ND
3'-Chloro-3'-deoxyxyloA	>125	>125	>125	>125	>125	>125	>125	>125	ND
3'-Azido-3'-deoxyAdo	>26	>26	>26	>26	>26	>26	>26	>26	26
3'-Azido-3'-deoxyaraA	>125	>125	>125	>125	>125	>125	>125	>125	ND
3'-Chloro-2',3'-dideoxyBVUrd	>125	>125	>125	>125	>125	>125	>125	>125	ND
3'-Azido-2',3'-dideoxylxyloU	>125	>125	>125	>125	>125	>125	>125	>125	ND
3'-Azido-2',3'-dideoxyxyloU	>125	>125	>125	>125	>125	>125	>125	>125	ND
Ribavirin	>125	>125	>125	>125	>125	>125	>125	>125	ND
Pyrazofurin	>32	>32	>32	>32	>32	>32	>32	>32	32
3-Deazaguanine	>125	>125	>125	>125	>125	>125	>125	>125	ND
Tubercidin	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4	>0.4	<0.4
(S)-DPHA	>125	>125	>125	>125	>125	>125	>125	>125	ND
C-c^{3}Ado	>125	>125	>125	>125	>125	>125	>125	>125	ND
(S)-HPMPA	0.8	1.3	0.6	0.7	1.5	0.6	1.0	2.5	52
2'-nor-cGMP	1.8	2.2	2.4	2.6	5.0	2.6	2.8	13.5	62
ACV	>125	>125	>125	>125	>125	>125	>125	>125	ND
AraA	>125	>125	>125	>125	>125	>125	>125	>125	ND
BVDU	>125	>125	>125	>125	>125	>125	>125	>125	ND
PFA	>125	>125	>125	>125	>125	>125	>125	>125	ND

[a] ND, Not determined.

against AV replication might be the viral DNA polymerase (15). For Vero cells infected with HSV type 1, we have recently found that (S)-HPMPA specifically inhibits viral DNA synthesis at concentrations (0.01, 0.1, 1, and 10 μg/ml) at which cellular DNA synthesis is not affected (E. De Clercq, T. Sakuma and R. Bernaerts, unpublished data). We are now exploring whether (S)-HPMPA is also specifically inhibitory to AV DNA synthesis.

Thus, (S)-HPMPA and 2'-nor-cGMP offer promise as broad-spectrum anti-DNA virus agents. The results of this investigation indicate that their activity spectrum extends to human AVs.

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