127

Activity of (*S*)-1-(3-Hydroxy-2-Phosphonylmethoxypropyl)Cytosine (HPMPC, Cidofovir) Against Murine and Human Polyomavirus Replication *In Vitro*

G. Andrei, R. Snoeck, M. Vandeputte and E. DeClercq
Rega Institute for Medical Research, K.U.Leuven, Leuven, Belgium

Polyomaviruses are double-stranded DNA viruses that are widely distributed in nature and responsible for progressive multifocal leukoencephalopathy (PML) and hemorrhagic cystitis mostly in immunocompromised patients. PML has been noted with increasing frequency due to the prolonged survival of AIDS patients, but has so far not proven amenable to therapy. Therefore, there is a need to develop molecules that are active against polyomavirus. Here we describe the activity of (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine (HPMPC, Cidofovir) and other acyclic nucleoside phosphonate derivatives against murine polyomaviruses (MN/RDE, PyPTA, Py2TA2 and LiD-1 strains) and human polyomaviruses (SV40/PML-1 and SV40/PML-2 strains). The assays with the murine polyomaviruses were performed in UC1-B cells. The highest selectivity index (S.I.) (ratio CC_{50}/IC_{50}) was obtained for HPMPC (S.I. ± 13) while for cyclic HPMPC, HPMPA, PMEA, PMEDAP and PMEG, the selectivity indexes were 7, 3, 4, 4, 4, respectively. None of the other drugs tested (acyclovir, ganciclovir, bromovinyldeoxyuridine or foscarnet) showed any activity. For the human polyomaviruses, the assays were performed in BS-C-1 cells. HPMPC had the best S.I. (30), followed by cyclic HPMPC, PMEG and HPMPA with a S.I. of 17, 6 and 4, respectively. PMEA, PMEDAP, as well as the other drugs tested, did not show any activity against human polyomaviruses. These results were confirmed in a virus yield assay for both murine and human polyomaviruses. A time of addition experiment showed that HPMPC was still active when added up to 16 hours post infection to murine polyomavirus-infected cells, while for human polyomaviruses addition of HPMPC could be delayed until 55 hours post infection. These results suggest that HPMPC may be a good candidate for the treatment of polyomavirus infections in the immunocompromised host.

128

Antiviral and antiinflammatory effects of pulmonary-delivered recombinant murine interferon-γ in a murine Coxsackievirus B3 model

N. Yamamoto, T. Masumoto, M. Shibamori, M. Ogura, T. Doi, N. Ise, and M. Kikuchi
Microbiological Research Institute, Otsuka Pharmaceutical Co., Ltd. 463-10 Kagasuno, Kawauchi-cho, Tokushima 771-01, Japan

The antiviral and antiinflammatory effects of recombinant murine IFN-γ (mIFN-γ; Hayashibara Biochem. Lab.; Lot. No. 04003, Activity; 6.5×10^5 IU/ml)were investigated using intra-tracheal administration, which is a new dosing route, and male mice with myocarditis induced by intraperitoneal inoculation of Coxsackievirus B3 (Nancy strain ATCC VR-30). The cytokine, mIFN-γ, was administered at doses of 100, 1,000 and 10,000 IU once daily for 4 days, starting from the day of inoculation, and the effects were compared with those of natural mIFN-α/β (Hayashibara Biochem. Lab., Lot. 005, Activity; 3.56×10^7 IU/ml), which was administered using the same treatment schedule to the same myocarditis model.

The survival time of the infected animals that were treated with mIFN-α/β did not increase dose dependently. In sharp contrast, mIFN-γ therapy increased survival time significantly ($p<0.01$) at dose of 1,000 and 10,000 IU, and the therapeutic effects of the high doses of 10,000 IU were marked, as evidenced by remarkable prolonged survival time ($p=0.0012$) and suppression of following; body weight loss ($p<0.01$), viral replication in heart ($p<0.01$), calcification ($p=0.0002$), and viral myocarditis ($p=0.0161$), which was determined by the severity of myocardial necrosis ($p=0.0170$). These findings suggest that intra-tracheal administration is an effective dosing route for IFN-γ when used to treat viral myocarditis.

129

STUDY OF THE MECHANISM OF ACTION OF 3(2H)-ISOFLAVENE ON POLIOVIRUS TYPE 2 INFECTION.

D. Genovese (1), S. Catone (1), G. Buttinelli (1), M. Elmi Farah (2), A. Gambacorta (3) and L. Fiore (1).
1) Lab. of Virology, Istituto Superiore di Sanita', Rome, Italy.
2) Dept. of Chemistry, "La Sapienza" University, Rome, Italy.
3) Dept. of Chemistry, III University, Rome, Italy.

3(2H)-isoflavene is a compound related to dichloroflavan with a broad spectrum of antipicornavirus activity. We have studied the effect of this compound on poliovirus type 2 Sabin. The presence of 3(2H)-isoflavene from the beginning of infection, or during the adsorption period only, prevented the shut-off of host translation and viral RNA and protein synthesis; furthermore the drug was not virucidal, did not protect virus infectivity from heat inactivation, and had no measurable effect on the binding of virus to cell, viral penetration and uncoating of the viral RNA; in contrast, the compound significantly reduced the infectivity of free viral RNA. These results strongly suggested that an early step of viral replication cycle may be selectively inhibited. We have attempted to elucidate the mechanism of inhibition of 3(2H)-isoflavene on poliovirus type2 Sabin infection in vitro by exploring the differences in nucleotide sequencing and studying the biological properties of mutants sensitive and resistant to this compound. Furthermore, recombinant viruses, carrying the isolated mutations found in the above mentioned mutants, have been constructed in order to clarify the level at which the drug interfere with poliovirus replication. The comparison of the sequences of resistant and dependent mutants with that of Sabin 2, show nucleotide changes in the 5' NCR and in the domain of 3CD protein. The results obtained from the biological characterization of the mutants and of the recombinants suggest that the compound interfere with a step that control the polyprotein translation.

130

Effect of Combination Therapy with Ribavirin and Human Interferon Alfa on Subacute Sclerosing Panencephalitis (SSPE) Virus in Hamsters

TOSHIMITSU TAKAHASHI, MITSUAKI HOSOYA and SHIRO SHIGETA
Department of Microbiology, Fukushima Medical College, Fukushima 960-12, Japan

The combination with ribavirin and interferon alfa had a synergistic effect in vitro. We studied the effect of combination therapy with two antiviral agents, i.e. ribavirin and interferon alfa on hamsters infected with subacute sclerosing panencephalitis (SSPE) virus. When antiviral agent was administered intracranially, ribavirin alone improved the survival of infected hamsters to 40% at a dose of 1 mg/kg/day and to 100% at a dose of 10 mg/kg/day, and interferon alfa alone improved it to 60% at a dose of 3×10^4 IU for two times. When administered both ribavirin at a dose of 1 mg/kg/day and interferon alfa at a dose of 3×10^4 IU, the combination therapy improved the survival to 100%. Thus, the combination therapy was more effective than the mono therapy, in vivo. The combination therapy did not increased the toxicity in uninfected hamsters by intracranial administration to compare with the ribavirin mono therapy. Intrathecal or intraventricular administration of two antiviral agents combined with ribavirin and interferon alfa shoud be explored for potential use in the treatment of patients with SSPE.