Oral Session III: Herpesviruses I and Poxviruses I

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Synthesis and Structure–Activity Aspects of Some Cyclic Cidofovir Peptidomimetic Prodrugs

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Cidofovir (HPMPC, Vistide®) is a broad-spectrum antiviral agent that is currently used to treat AIDS-related CMV retinitis. Cidofovir is of particular interest as a potential therapy for orthopox virus infections but is limited by poor oral bioavailability, prompting efforts to create prodrug modifications. We previously reported the synthesis and biological evaluation of cyclic cidofovir phosphonate ester prodrugs incorporating an amino acid attached via an ethylene glycol linker, or else an X-Ser dipeptide C-ester connected via the serine side-chain hydroxyl group. Both types of prodrugs were activated by cellular and tissue homogenates to release the active parent drug, and the ethylene glycol-linked prodrug was 4× more active than ganciclovir in a HCMV plaque reduction assay. However, only the directly conjugated dipeptide prodrugs exhibited enhanced transport properties versus cidofovir. We have now examined structure–activity effects of varying the stereochemistry of the amino acids comprising the dipeptide auxiliary. We have also synthesized a cognate prodrug containing a free serine carboxyl group. The prodrugs were evaluated for pH-dependent and intestinal cell homogenate stability, antiviral activity and transport enhancement.

doi:10.1016/j.antiviral.2007.01.023

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Synthesis and Antiviral Activity of 1-(*S*)-[3-Hydroxy-2-(Phosphonomethoxy)Propyl]-5-Azacytosine and its Ester Prodrugs

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Investigation of new acyclic nucleotide analogs as potential antivirals resulted in development of analogs with 5-azacytosine moiety (Krečmerová et al., submitted for publication). 1-(*S*)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine (1), a 5-azacytosine analog of cidofovir exerts a strong activity against adenoviruses, poxviruses, herpes simplex viruses, VZV and CMV in cell cultures. For all these DNA viruses, 1 showed a 2–16-fold higher antiviral selectivity index compared to cidofovir. Transformation of 1 to appropriate ester prodrugs was carried out on the level of its cyclic phosphonate 2 (Fig. 1). Several types or structurally diverse esters were synthesized: alkyl (octadecyl 3a), acyloxyalkyl (pivaloyloxymethyl 3b) and alkoxyalkyl (Kern et al., 2002) (e.g. hexadecyloxyethyl 3c). The most active prodrug was found ester 3c. The development of other prodrugs is under way.

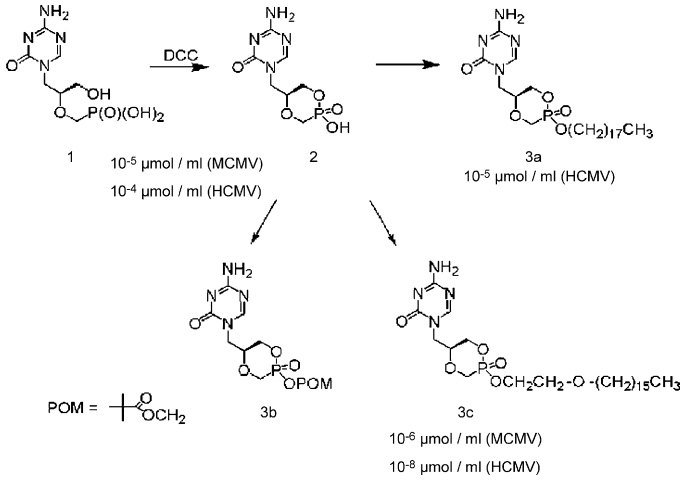

Fig. 1.

Acknowledgements: *Financial support*: Research project of IOCB Acad. Sci. Czech Republic # Z4 055 0506, Centre for New Antivirals and Antineoplastics 1M0508, NIH 1UC1 AI062540-01, René Descartes Prize-2000-grant HPAV-2002-100096, the Grant Agency Acad. Sci. (# 1QS 400550501) and Gilead Sciences.

References

Krečmerová, M., Holý, A., et al. J. Med. Chem., submitted for publication.

Kern, E.R., et al., 2002. Antimicrob. Agents Chemother. 46, 991–995.

doi:10.1016/j.antiviral.2007.01.024

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In Vivo Antiviral Activity of 1-(*S*)-[3-Hydroxy-2-(Phosphonomethoxy)Propyl]-5-Azacytosine and its Cyclic Form

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1-(*S*)-[3-Hydroxy-2-(phosphonomethoxy)propyl]-5-azacytosine [(*S*)-HPMP-5-azaC] emerged as a potent and selective inhibitor of several DNA viruses, including herpesviruses [i.e. herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2, varicella-zoster virus (VZV), human herpes virus 6 (HHV-6) and cytomegalovirus (CMV)], adenovirus type2 (Ad2) and poxviruses [i.e. vaccinia virus (VV), cowpox virus (CPV) and orf virus]. The antiviral activity of (*S*)-HPMP-5-azaC was comparable to that of the reference drug (*S*)-HPMPC (cidofovir) against HSV-1, HSV-2 and vaccinia virus, and two to seven-fold higher than cidofovir against VZV, HCMV, HHV-6 and Ad2. The cyclic derivative of (*S*)-HPMP-5-azaC (i.e. cHPMP-5-azaC) was also able to inhibit the replication of these DNA viruses. We have now evaluated the in vivo activity of HPMP-5-azaC and cHPMP-5-azaC against HSV-1, HSV-2, VV and CPV using two murine lethal challenge models. Five NMRI mice per group were infected with a lethal dose of HSV-1 or HSV-2 (intraperitoneal infection) or VV or CPV (intranasal infection) and treated subcutaneously with (*S*)-HPMP-5-azaC, cHPMPC-5-azaC, (*S*)-HPMPC or cHPMPC once daily from day 0 to day 4 post-infection at a dose of 50 mg/kg. The virus dose used in each experiment resulted in 100% mortality in the untreated animals. (*S*)-HPMP-5-azaC and cHPMP-5-azaC proved to be as effective as, respectively, (*S*)-HPMPC and cHPMPC in reducing mortality, affording 100% survival. Furthermore, a single dose of 50 mg/kg of each of the four compounds administered on day 0 was sufficient to provide full protection. Oral prodrugs were synthesized [i.e. alkyl (e.g. octadecyl), acyloxyalkyl (pivaloyloxymethyl) and alkoxyalkyl (e.g. hexadecyloxyethyl)], the latter being the most active prodrug in vitro. The in vivo activities of these prodrugs are currently under investigation. These findings warrant further development on this new class of acyclic nucleoside phosphonates.

doi:10.1016/j.antiviral.2007.01.025

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Isolation and Characterization of an Helicase-Primase Inhibitor (HPI)-Resistant HSV-1 Mutant in Tissue Culture and a Mouse-Skin Model

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Nucleoside analogues currently dominate HSV therapy and drug resistance is readily selected in cell culture (>10^{-4}) but rarely occurs in patients. Common resistant variants have defective thymidine kinase; although these mostly grow normally in tissue culture, they are often attenuated.

HPI, e.g. BAY 57-1293 are a newer class of antivirals, which have superiority over nucleoside analogues in animal infection models. BAY 57-1293-resistance could be selected by single passage in the presence of inhibitor. For example, BAYr1 is 70-fold resistant to BAY 57-1293 and occurred at a frequency of 10^{-6} in the well-characterized HSV-1 strain, SC16. There are previous reports that drug-resistant mutants of HSV-1 selected against BAY 57-1293 or other HPI have slower or near wild-type growth rates in vitro. However, we showed consistently that BAYr1 replicated faster in cell culture than its parent, SC16.

BAYr1 was fully pathogenic in a murine skin-infection model according to all the clinical parameters, including latency. BAYr1 was found to have two substitutions in the helicase protein (UL5: A4V and K356Q). Marker transfer revealed that K356Q alone is responsible for 70-fold resistance and faster growth in culture. Our results with BAYr1 support and extend previous reports: an HSV-1F mutant resistant to BAY 57-1293 (K356N) gave near wild-type mortality in a mouse survival test. Two mutants from HSV-1 KOS resistant to BILS 22 BS, a different HPI (K356N or G352V) grew normally in culture and were pathogenic in animal models. Our results and these two reports will be discussed in the relation to our recent surprising finding that some laboratory and clinical isolates of HSV-1 contain HPI-resistant mutants at high frequency.

doi:10.1016/j.antiviral.2007.01.026

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Evidence For In Vivo Inhibition of CMV Infection by the Quinazoline Class Protein Kinase Inhibitor Gefitinib

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Gefitinib (Iressa®) is a chemotherapeutic agent that exhibits activity against human and animal cytomegaloviruses (CMVs). We examined the activity of this agent against the guinea pig cytomegalovirus (GPCMV) both in vitro and in an in vivo disease model. In plaque reduction assays, gefitinib exhibited an IC_{50} of 3.3 μM, lower than the IC_{50} demonstrated against human CMV. The efficacy of gefitinib was next evaluated in an immunosuppression CMV disease model in outbred guinea pigs. Seronegative animals were treated intraperitoneally (i.p.) with cyclophosphamide, at a dose of 300 mg/kg at day −1 and 100 mg/kg at day +7, relative to i.p. challenge with an eGFP-tagged recombinant GPCMV, administered at a sublethal dose of 2×10^5 pfu. Beginning at day 1, guinea pigs were treated either with oral placebo ($n = 8$), or with either cyclic cidofovir at a dose of 20 mg/kg i.p. once weekly ($n = 8$), or gefitinib at a dose of 100 mg/kg once daily by gavage ($n = 8$). Antiviral therapy had a significant impact on weight loss. In placebo-treated animals, mean weights decreased in surviving animals by 17% at the endpoint of the experiment, compared to a 7% decrease in gefitinib-treated animals, and a 15% increase in