Oral Session 5: Herpesvirsues and Poxviruses

32
Role of Angiogenesis and Wound Repair Factors in the Acceleration of Allograft Rejection by Cytomegalovirus

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33
Design, Synthesis and Evaluation of Novel Anti-VZV BCNAs

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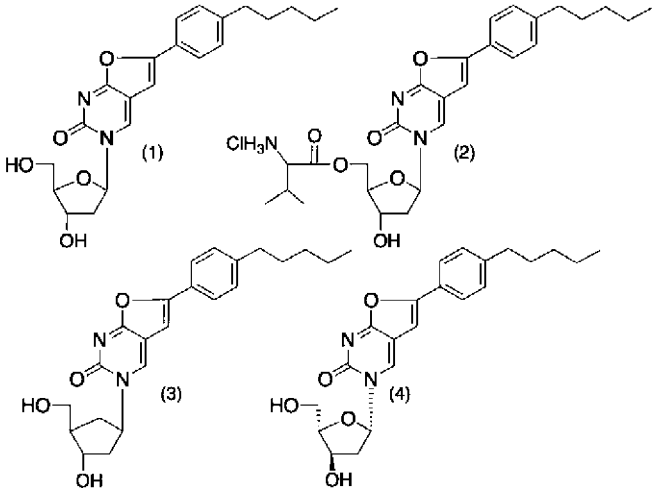
The bicyclic nucleoside analogues (BCNAs) are a family of exquisitely selective anti-VZV agents developed in our laboratories. The lead compound Cf1743 (**1**) has recently entered phase 1 clinical trials as its 5′-valyl ProDrug (FV100) (**2**). We herein report modifications in the sugar region of the lead (McGuigan et al., 2007). In particular, we describe the design, synthesis and evaluation of the carbocyclic analogue of (**1**) and also the L-enantiomer of (**1**), compounds **3** and **4**, respectively. Besides antiviral data, we will present VZV TK inhibition data and also molecular modelling studies of key agents with the VZV-encoded nucleoside kinase.



Reference

McGuigan, C., et al., 2007. J. Antimicrob. Chemother. 60, 1316–1330.

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34
Maribavir Inhibits the Replication of Human Herpesvirus 6 and the Activity of the U69 Protein Kinase

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Infections with the B variant of human herpesvirus 6 (HHV-6) are self-limiting and occur in almost all children very early in life. In immunocompromised hosts, reactivation of either the A or B variant can cause serious disease, particularly those receiving hematopoietic cell transplants. Drugs with improved efficacy and good toxicity profiles are needed to treat these infections in transplant patients. We reported previously that maribavir (MBV) was a poor inhibitor of HHV-6 replication. This lack of activity was difficult to explain since MBV was a good inhibitor of human cytomegalovirus (HCMV) replication, and the UL97 protein kinase targeted by this drug in HCMV was conserved in HHV-6 (U69). Recent results from our laboratory suggested that the inactivation of the RB tumor suppressor by UL97 was an important function and that this did not occur in the presence of MBV. This result was consistent with the reduced activity of MBV against HCMV in dividing cells where the stimulation of the cell cycle by RB was less important. We reasoned that the poor activity observed with MBV against HHV6 might reflect the fact that the assays were conducted in rapidly dividing lymphocytes. To test this hypothesis, the activity of MBV was reevaluated against the GS strain of HHV-6A in HSB-2 cells with reduced serum to slow the growth of the lymphocytes. Under these conditions, MBV exhibited increased activity with EC_{50} values of approximately 15 μM, while the EC_{50} values for the cidofovir controls were unaffected. To confirm these results, we developed a surrogate assay for activity of the U69 protein kinase based on its kinase-dependent inhibition of nuclear aggregation. In this assay, MBV proved to be a good inhibitor of U69 activity and suggested that this kinase was also targeted by the drug. These results suggest that MBV is a good inhibitor of HHV-6 replication and the target of the drug is likely the U69 protein kinase. We conclude that the spectrum of activity of MBV includes Epstein-Barr virus, HCMV, and HHV-6 and may be useful in the treatment of these infections.

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35
Antiviral Potency of ST-246 on the Production of Enveloped Orthopoxviruses and Characterization of ST-246 Resistant Vaccinia, Cowpox and Camelpox Viruses

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ST-246 is a highly potent and orally bioavailable anti-orthopoxviral molecule that targets the F13L protein of vaccinia virus (VACV). Its in vitro antiviral effect against VACV,

camelpox virus (CMLV) and cowpox virus (CPV) have raised questions about potential differences in VACV, CPV and CMLV replication cycles and the possible presence of other targets. Based on these data, we investigated the relative quantity of enveloped virus versus non-enveloped virus in the medium and cell lysates using density gradients performed on [methyl-^{3}H]-thymidine labeled VACV, CPV and CMLV infected cells, either in the absence or presence of ST-246. We then characterized ST-246 resistant orthopoxviruses which were selected following increasing concentrations of ST-246 for approximately 17 passages. Analysis of cesium chloride gradient fractions indicated that most of the viruses produced during a productive VACV, CPV and CMLV infection were enveloped. The spread of VACV appeared to involve both intra- and extracellular enveloped forms. In contrast, CPV and CMLV produced few extracellular enveloped forms and seemed to propagate via intracellular infectious particles. These data indicated a difference in the way of propagation of CMLV/CPV and VACV. It was also clear that the antiviral activity of ST-246 was due to an interference with the formation of enveloped forms and that the discrepancy in the inhibitory effect of ST-246 on orthopoxvirus replication was more likely due to differences in orthopoxvirus spread. The IC_{50}s determined for each ST-246 resistant virus mutant were at least 100–2000-fold higher than those of the wild-type viruses. Sequencing of the F13L genes of both resistant and wild-type VACV, CPV and CMLV strains is currently ongoing. We have demonstrated the antiviral potency of ST-246 on the production of enveloped orthopoxvirus particles and confirmed differences in VACV, CPV and CMLV replication cycles. ST-246 resistant viruses have been plaque purified and the determination of mutations induced by ST-246 in the F13L gene is currently under investigation.

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36

Vaccinia DNA Polymerase is Profoundly Inhibited by Cidofovir and (*S*)-HPMPA Incorporated into the Template Strand

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Cidofovir (CDV) and (*S*)-9-[3-hydroxy-(2-phosphonomethoxy)propyl]adenine (HPMPA) are analogs of dCMP and dAMP, respectively, and are effective inhibitors of poxvirus replication. However, the precise mechanism by which these drugs inhibit viral growth remains unclear. We have previously used vaccinia virus as a model system to show that the diphosphoryl metabolites of these drugs are substrates for vaccinia DNA polymerase, but once incorporated into the penultimate 3′-end of the primer strand inhibit both the polymerase and 3′-to-5′ exonuclease activities. Interestingly, although HPMPA exhibits a much lower EC_{50} than CDV, HPMPApp is not nearly as effective an inhibitor of primer extension and exonuclease activity in vitro compared to CDVpp. This suggests that other mechanism(s) must account for the differences in relative activities of the two drugs. To investigate these mechanisms, we examined the effects of CDV and HPMPA on vaccinia DNA polymerase activity when incorporated into the template strand. These templates were prepared using a two-step enzymatic method. First, oligonucleotide-primed templates were prepared containing a single dGMP or dTMP residue (to direct CDV or HPMPA incorporation, respectively) and multiple dUMP residues. Primer extension was used to incorporate each drug, and then the dUMP-containing strand was degraded using uracil DNA glycosylase. Labeled primers were then annealed to the newly extended strands and we tested whether vaccinia DNA polymerase could extend these primers back across the drug residue. We found that although the correct nucleotide could be incorporated opposite the drug lesion, further extension by the DNA polymerase was blocked. Control templates, containing either dCMP or dAMP at the same sites, did not show this block. These results suggest that although primer extension is slowed by the incorporation of these drugs, the profound effects on DNA replication are more likely caused by drug incorporated into the template strand. HPMPApp is a much better substrate for vaccinia DNA polymerase than is CDVpp, but this makes it the more effective drug because more HPMPA is then incorporated into the template strand.

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37

Development of a Model for the Study of Antivirals Against Molluscum Contagiosum Virus (MCV)

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Molluscum contagiosum virus (MCV) and variola virus (VARV) are the only two poxviruses that are specific for humans. VARV has been eradicated from the human population through extensive programs of vaccination. MCV is present worldwide and is directly passed by direct skin contact to produce cutaneous and, rarely, mucosal lesions. It occurs predominantly in preadolescent children, sexually active adults, and in individuals with impaired immunity. The study of MCV has been hampered by the lack of an in vitro system that allows virus replication. We describe here the growth of MCV in 2D (two dimensional) and 3D cultures of primary human keratinocytes (PHKs). PHKs were isolated from neonatal foreskins and used to prepare monolayer cultures and organotypic raft cultures, which are able to mimic fully differentiated skin. Fresh lesions obtained from preadolescent children were used to recover the MCV. Organotypic epithelial raft cultures were infected with different MCV fresh isolates and after a period of approximately 25 days were processed for histological examination. A typical cytopathic effect characterized by the appearance of huge infected cells, with internal organelles dislocated and obliterated by a large intracytoplasmic