157

Novel Inhibitors of Human Cytomegalovirus (HCMV) Cascade Gene Expression

Jun Wu, Joe O'Neill and Miguel S. Barbosa, Virology Group, Signal Pharmaceuticals, Inc., San Diego, California 92121, U. S. A.

HCMV infection is a major cause of morbidity and mortality in immunocompromised patients. Problems with side effects and viral resistance of current therapy have made development of new and more effective anti-HCMV drugs a priority in antiviral drug research. HCMV infection of permissive cells in culture leads to an ordered sequential expression of viral genes which are divided into three kinetic classes: immediately-early (IE), early (E) and late (L). IE gene expression is essential for viral replication. These regulatory proteins present attractive targets for antiviral therapy since their inhibition should prevent viral replication without affecting the host-cell machinery. We established novel cell based assays to identify compounds that inhibit the HCMV cascade gene expression. Several compounds were identified and characterized. Compounds with antiviral activity were identified. Analysis of the inhibitor effect on the viral gene transcription characteristic of HCMV life cycle showed that the compounds acted by inhibiting the progression from IE to E phases of gene expression and viral production. The mechanism of action will be discussed.

158

Inhibition of Proteinase Dependent Processing of the Scaffold Proteins of HCMV Capsid by Monocyclic β-Lactams

L. Lagacé, F. Dô, E. Malenfant, R. Déziel and M. G. Cordingley
Bio-Méga Research Division Boehringer Ingelheim Canada Ltd, 2100 Cunard, Laval, P. Québec, Canada H7S 2G5.

Herpesvirus capsid assembly proceeds via a non DNA containing capsid with an internal scaffold core that is not present in mature virions. The scaffold is composed of the viral protease and assembly protein (AP) and proteolytic processing of these proteins is essential for capsid maturation in order to produce infectious virions. In human cytomegalovirus (HCMV), the protease is expressed as a precursor protein that undergoes autocatalytic cleavage to free the catalytic domain of the enzyme located in the N-terminal 256 amino acids. Our medicinal chemistry efforts towards the development of inhibitors of HCMV protease have generated a series of monocyclic β-lactam inhibitors of the catalytic domain. We have evaluated the activity of these inhibitors on the processing of the protease precursor as well as the processing of the assembly protein using cells transfected with the protease and AP, and in cells infected with HCMV. These protease inhibitors exhibited concentration dependant inhibition of the protease precursor processing as well as inhibition of the AP processing by the protease catalytic domain in transfected cells. When evaluated in infected cells, accumulation of the protease precursor and the assembly protein precursor could be observed in cells treated with these inhibitors. The results of this study suggest that these β-lactam inhibitors can inhibit both forms of HCMV protease in transfected and in infected cells. Experiments are in progress to correlate the inhibition of protease and assembly protein processing with effects on DNA encapsidation and maturation of capsids to form infectious virions following treatment with these inhibitors.

159

Modulation of HCMV gene expression by isoquinolines.

J. Bedard[1], A. Copsey[2], T. Stamminger[3], D.L. Taylor[2], A.S. Tyms[2], L. Chan[1], T. Stefanac[1], T. Bowlin[1], and R.F. Rando[1]
[1]BioChem Therapeutic Inc. 275 Armand-Frappier, Laval, H7V 4A7 Canada
[2]MRC Collaborative Center, 1-3 Burtonhole Lane, Mill Hill, London, NW7 1AD, UK
[3]Institut fur Klinische und Molekulare Virologie, Erlangen, Germany

The anti-HCMV potential of isoquinoline analogues was discovered using a plaque reduction assay with some of the more potent analogues in this series having IC_{50}s in the 0.005 μg/ml range with selective indices >100. These molecules were found to exhibit their highest antiviral activity when added to infected cells within 30 hours post-infection indicating a target acting at an immediate-early to early stage of virus infection. Utilizing virus specific MAbs and indirect immunofluorescence we determined that the isoquinolines were not interfering with virus infection but rather de novo synthesis of proteins expressed early in the infectious cycle was altered. We also determined, using a cell-based assay in which the HCMV MIEP promoter was linked to the luciferase gene, and expressed constitutively in HeLa cells, that the drug candidates were inhibiting transcription driven by the MIEP promoter. Plasmid transfection experiments using various subfragments of the MIEP linked to the CAT gene helped identify nuclear transcription factors affected by the presence of drug in the culture medium. In this regard we were able to decrease transcription enhanced by the 18 and 19 bp repeat elements found in the MIEP up to 80% at non-toxic drug concentrations. The correlation between reduced virus production in culture assays and the ability of the isoquinoline analogues to modulate important transcription factors necessary for the efficient replication of the virus, suggests that the targeting of such transcription factors may prove valuable in the design of novel antiviral agents.

160

Pharmacokinetic Studies of Naphthyridine Analog HCMV Inhibitors and Discovery of Substituted Isoquinoline Analogs as Metabolically Stable Anti-HCMV Agents.

H. Jin, L.Chan, T. Stefanac, Z. Hu, Yanmin Chen, Odette Bélanger, J. Bedard, Suzanne May and Annie St-Pierre. BioChem Therapeutic Inc., Laval, Québec, Canada H7V 4A7

Our recent effort in anti-HCMV program led to the identification of two novel classes of potent and selective anti-HCMV agents, the 1,6-naphtyridines and the isoquinolines. We were also encouraged by the fact that both lacked features that may be associated with poor oral bioavailability. However, during preliminary pharmacological evaluation, it was found that these compounds were not stable to the first pass metabolism. The metabolite was identified and found to be inactive in HCMV assay. Blocking the site of metabolism also abolished antiviral activity. However, based on SAR in naphthyridines and isoquinolines, we designed isoquinoline analogs and found that these compounds were much more metabolically stable both *in vitro* and *in vivo* and yet maintained anti-HCMV activity. The potential of these compounds for development will be evaluated.