131

Nucleotide Analogs that Induce Cellular Production of Antimicrobial Proteins

Radhakrishnan Iyer[1,*], John Coughlin[1], Seetharamaiyer Padmanabhan[1], Brent Korba[2], Sua Myong[3], Bud Tennant[4], John Morrey[5]

[1] *Spring Bank Pharmaceuticals, Inc., Milford, USA;* [2] *Georgetown University, Rockville, USA;* [3] *University of Illinois, Urbana-Champaign, USA;* [4] *Cornell University, Ithaca, USA;* [5] *Utah State University, Logan, USA*

Innate immunity plays a significant role in the antimicrobial defense against viruses, as well as, prokaryotic and eukaryotic pathogens. Cell-mediated immune response to viral infections occurs through cellular pathogen recognition receptors (PRRs) that bind to pathogen-associated molecular patterns (PAMPs) and activation of intracellular signaling pathways. Recently, peptides homologous to beta chain of hemoglobin (Hb-beta) have been reported to play an important role in the cellular antimicrobial defense. The Hb-beta, also called extra-erythrocytically expressed hemoglobin [EEEH], is produced by macrophages, alveolar epithelium, and vaginal tissues. Interestingly, AMPs homologous to EEEH are known to be upregulated in the gills and skin epithelium of fish and have potent antiparasitic and antibacterial activity. We have recently discovered that the orally bioavailable dinucleotide compound SB 44 (a prodrug of the anti-HBV compound SB 40) induces increase in plasma EEEH in HBV transgenic mice which correlated with its anti-HBV activity. Thus, for example, orally administered SB 44 at 1, 5, 10, and 100 mg/kg showed a dose-dependent increase in plasma EEEH that correlated with dose-dependent reduction of liver HBV DNA. The sublingual administration of a polyether formulation of the dinucleotide – SB 40 – also caused sustained increase in plasma EEEH in woodchucks. The presence of EEEH with a MW of 15 kDa was confirmed by SDS gel electrophoresis followed by proteomic analysis. We therefore hypothesize that a component of the antiviral action of the dinucleotides SB 40 and SB 44 involves the upregulation of the EEEH that is mediated through the host innate immune system. Furthermore, we believe that EEEH is a potential biomarker that indicates the presence of an active innate immune defense against viral infections.

doi:10.1016/j.antiviral.2010.02.441

132

Synthesis and Antiviral Evaluation of 3-(2,3-Dihydroxypropyl)furo[2,3-*d*]pyrimidin-2(3*H*)-ones

Zlatko Janeba[1,*], Antonín Holý[1], Robert Snoeck[2], Graciela Andrei[2], Erik De Clercq[2], Jan Balzarini[2]

[1] *Institute of Organic Chemistry and Biochemistry AS CR, Prague, Czech Republic;* [2] *Rega Institute for Medical Research, K.U.Leuven, Leuven, Belgium*

Bicyclic nucleoside analogues (BCNAs) are potent and selective inhibitors of varicella-zoster virus (VZV) replication. The antiviral compound Cf 1743 is one of the most potent and selective antiviral agents and its orally bioavailable prodrug FV100 (5′-valine ester of Cf 1743) is currently in Phase II clinical development for the treatment of herpes zoster (shingles). SAR studies of the BCNAs showed that sugar modifications are not well tolerated for maintaining potent anti-VZV activity. Thus, while 2′-deoxyribosides are potent and selective anti-VZV agents, their ribo- and arabino-analogues are significantly less active. Furthermore, 2′,3′-dideoxy analogues and N-3 alkyl derivatives showed poor activity against VZV but they surprisingly exhibited activity against human cytomegalovirus (HCMV) infection. Replacement of the sugar at the N-3 position of the BCNAs by the (2-hydroxyethoxy)methyl group (as in the antiherpes drug acyclovir) afforded compounds with weak activity against both VZV and HCMV. Although phosphorylation of the BCNAs by the VZV-encoded thymidine kinase (TK) is a prerequisite for their anti-VZV activity, acyclic nucleotide analogues (2-phosphonomethoxyethyl derivatives) of the furo[2,3-*d*]pyrimidin-2(3*H*)-ones were not active at sub-toxic concentrations. To help further elucidate the mechanism of antiviral action of the BCNAs, novel 3-(2,3-dihydroxypropyl)furo[2,3-*d*]pyrimidin-2(3*H*)-ones were synthesized. The target compounds were prepared by the Sonogashira coupling of various 1-alkynes with 1-(2,3-dihydroxypropyl)-5-iodouracil, followed by in situ Cu(I)-promoted intramolecular cyclization. The activity of these compounds against VZV and HCMV will be reported.

Acknowledgements: IOCB project OZ40550506; No. 1M0508, No. 1QS400550501; Gilead Sciences and IOCB Research Center.

doi:10.1016/j.antiviral.2010.02.442

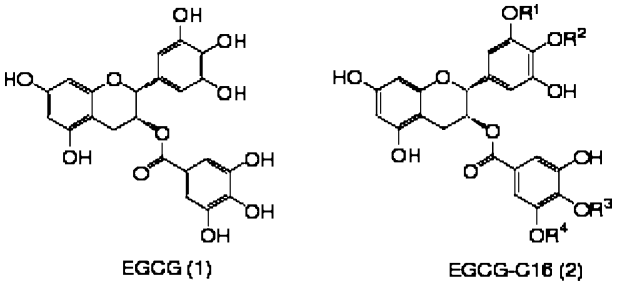

Fig. 1. Chemical structure of EGCG (**1**) and EGCG-C16 (**2**). **2** is a mixture of four regio-isomers (**2a–d**). **2a**: R^2=R^3=R^4=H, R^1=$CO(CH)_{14}CH_3$; **2b**: R^1=R^3=R^4=H, R^2=$CO(CH)_{14}CH_3$; **2c**: R^1=R^2=R^4=H, R^3=$CO(CH)_{14}CH_3$; **2d**: R^1=R^2=R^3=H, R^4=$CO(CH)_{14}CH_3$. The ratio of each regio-isomer **2a**:**2b**:**2c**:**2d** is 38:35:7:20, respectively.

133

Combating Drug-resistant Influenza Viruses with Novel Green Tea Catechin Derivatives

Kunihiro Kaihatsu[1,*], Hiroyo Matsumura[1], Shuichi Mori[1], Chiharu Kawakami[2], Hideshi Kurata[2], Nobuo Kato[1]

[1] *Osaka University, Ibaraki, Japan;* [2] *Yokohama City Institute of Health, Yokohama, Japan*

Seasonal influenza epidemic and pandemic outbreaks cause significant disease burdens and mortality in humans. The 1918 Spanish flu pandemic was caused by an influenza A (H1N1) virus and resulted in the death of millions of people worldwide. With the ever-present threat of an influenza pandemic, antiviral compounds are in great demand, yet such drugs for the treatment and prophylaxis of influenza are limited. A neuraminidase (NA) inhibitor, oseltamivir phosphate, is the most commonly used antiviral drug. However, reports found that some new seasonal influenza viruses possess resistance to oseltamivir phosphate.

(−)-Epigallocatechin-3-*O*-gallate (EGCG; **1**), a major green tea component (*Camellia sinensis*), has been recognized to possess antiviral activities. Recently, we have reported a method to synthesize EGCG-fatty acid monoesters using lipase-catalyzed transesterification and confirmed that EGCG-fatty acids showed improved influenza virus inhibitory effect in an alkyl length dependent manner (Mori et al., 2008).

Here we studied the anti-influenza virus activity of EGCG (**1**, Fig. 1) and EGCG-C16 (**2**, Fig. 1) on drug-resistant influenza A (H1N1)

viruses isolated in Yokohama during 2007–2008. As a result, it was appeared that **2** completely inhibited the infection of their drug-resistant viruses. Further, the virus inhibition activity of **2** was found to be 20-fold relative to **1**. This unique virus inhibitory action can be utilized to inhibit a broad spectrum of influenza viruses.

doi:10.1016/j.antiviral.2010.02.443

134

Small Molecule Therapeutics of Viruses of Families *Bunyaviridae* and *Arenaviridae*

Marcela Karpuj[1,*], Darci Smith[2], Brenna Kelley-Clarke[3], Andrea Stossel[2], Anna Honko[2], Sean Broce[4], Nessie Van Loan[4], Emma Harrell[4], Colm Kelleher[4], Jaisri R. Lingappa[3], William Hansen[4], Clarence R. Hurt[4], Lisa Hensley[2], Vishwanath R. Lingappa[4]

[1] *CUBRC, Inc., Buffalo, USA;* [2] *Virology Division, USAMRIID, Ft Detrick, USA;* [3] *Department of Global Health, University of Washington, Seattle, USA;* [4] *Prosetta Bioconformatics, Inc., San Francisco, USA*

The nucleoprotein of Rift Valley Fever Virus (RVFV NP), a member of family *Bunyaviridae*, and of Lassa Fever Virus (LASV NP), a member of family *Arenaviridae* both of which have helical capsids, have been expressed in a system for cell-free protein synthesis (CFPS). Assembly was assessed by velocity sedimentation on sucrose density gradients and occurs under conditions previously demonstrated to assemble icosahedral capsid-related structures for multiple virus families. The nucleoprotein assembly pathways of these helical capsid viruses appear distinct from each other as well as from those of the several families of icosahedral capsid viruses studied previously. Preliminary electron microscopic studies confirm an appearance of nucleoprotein assembled by CFPS that is similar to irradiation-inactivated, detergent treated, authentic RVFV and LASV, and markedly different from the structures formed for icosahedral capsid viruses, as would be expected. ELISA screens have been devised for identification of small molecules blocking these assembly pathways. Hits from these screens have been validated by plaque reduction assessment of live virus in cell culture. Partial overlap was observed between the compounds active against RVFV and those active against LASV, with some compounds active against one but not the other, and other compounds active against both. One possible explanation for these findings is that these viral families share a requirement for some host factors. Studies are proceeding on putative host target identification using drug column affinity chromatography of extracts prior to CFPS, on dissection of mechanism of drug action by analysis of the products of CFPS in the presence of compounds, and on structure activity relationship optimization to enhance potency and diminish toxicity.

doi:10.1016/j.antiviral.2010.02.444

135

Efficacy of N-methanocarbathymidine Against Herpes Simplex Virus is Cell Cycle Dependent

Kathy Keith[1,*], Emma Harden[1], Rachel Gill[1], Victor Marquez[2], Earl Kern[1], Mark Prichard[1]

[1] *The University of Alabama School of Medicine, Birmingham, USA;* [2] *National Cancer Institute, Frederick, USA*

(North)-methanocarbathymidine (N-MCT) is a conformationally locked analog of thymidine that is a good inhibitor of herpes simplex virus (HSV) and orthopoxvirus replication in vitro and in vivo. This compound is phosphorylated by the thymidine kinase (TK) encoded by herpes simplex virus and also by the TK homologs encoded by the orthopoxviruses. However, the mechanism of action is complex and other cellular kinases also likely play a role in its metabolic activation in infected and uninfected cells. Isolates of HSV that are resistant to acyclovir are also comparatively resistant to N-MCT which was expected since mutations that reduce TK activity also reduce the activation of both compounds. However, the efficacy of the compound against acyclovir-resistant isolates varied widely depending on the state of the primary human foreskin fibroblast (HFF) cells used in these studies. When HFF cells were seeded 3 days prior to infection, the compound inhibited TK deficient strains of HSV-1 with EC_{50} values of 3–10 μM, but if cells were seeded two days prior to infection the compound was much less effective and had EC_{50} values of 48–66 μM. A similar effect was observed against TK deficient strains of HSV-2. Significant differences were not observed in the efficacy of cidofovir controls. The differential efficacy is likely related to the cell cycle since most of the cells are in the S phase of the cell cycle 2 days after the cells are seeded, and most are in G_1/G_0 by 3 days. It is unclear why the compound would be less effective in dividing cells; it is possible that increased levels of dTTP during S phase might compete with N-MCT triphosphate for incorporation by the viral DNA polymerase. Nonetheless, this observation is intriguing and could potentially be useful as the antiviral and the antitumor properties of this compound are of significant interest.

Acknowledgement: Supported by contract N01-AI-30049 from the NIAID, NIH.

doi:10.1016/j.antiviral.2010.02.445

136

Synthesis and Antiviral Activity of Adamantyl Modified Nucleoside Phosphonates: Analogs of Cidofovir

Yuri Klimochkin*, Alexander Reznikov, Michael Skomorokhov, Eugene Golovin

Samara State Technical University, Samara, Russia

Cidofovir (HPMPC, CDV, 1-(S)-[3-hydroxy-2-(phosphonomethoxy)propyl]cytosine) is a potent and selective anti-DNA virus agent. Cidofovir suppresses the in vitro growth of all human and animal DNA viruses thus for examined. In the ongoing search for new cidofovir analogues and derivatives, accruing attention is given to the development of neutral ester prodrugs to enhance oral absorption and improve pharmacological parameters. In the paper, we described the synthesis of novel nucleoside phosphonates modified by adamantyl moiety: R = $AdCH_2$, $AdCH_2CH_2$, $AdCH_2CH_2CH_2$, $AdOCH_2CH_2$, $AdOCH_2CH_2CH_2$, $AdOCH_2CH_2CH_2CH_2$, $AdOCH_2CH_2OCH_2CH_2$, 3-Et-$AdCH_2$, 3-Et-$AdCH_2CH_2$, 3-Et-$AdCH_2CH_2CH_2$, 3-Et-$AdOCH_2CH_2$, 3-Et-$AdOCH_2CH_2CH_2$, 3-Et-$AdOCH_2CH_2CH_2CH_2$, 3-Et-$AdOCH_2CH_2OCH_2CH_2$. This way of modification could allow developing new therapeutic agents having high level of bioavailability and can be able to act on two or more stages of reproductive cycle of DNA viruses.

Acknowledgments: The research was supported by the Russian Federal Agency on Science and Innovations and Russian Federal Education Agency (02.552.11.7077, 02.740.11.0394 and P706 (12.08.2009).