as follows: HBB > oxoglaucine > ribavirin > disoxaril > PTU-23 > arildone > S7.

doi:10.1016/j.antiviral.2008.01.074

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Broad Anti-Infective Activity of Viracea, An *Echinacea*-derived Product

Tracy L. Hartman [1,*], Karen M. Watson [1], Lu Yang [1], Meryl J. Squires [2], Robert W. Buckheit Jr. [1]

[1] ImQuest BioSciences, Inc., Frederick, USA; [2] Merix Pharmaceutical Corporation, Barrington, USA

Echinacea is a well-studied herb noted for stimulating the human immune system. There is evidence that *Echinacea* has the potential to treat a broad range of infectious diseases. Viracea, a proprietary extraction and formulation of *Echinacea* is presently marketed as RELEEV^{TM}, a commercial OTC product for the treatment of cold sores. Preclinical data indicates this product is active against HSV-1 and HSV-2. We have evaluated the broad based anti-infective properties of these products, including RELEEV and Viracea 2,4, an unfractionated product comprised of the aerial parts of *Echinacea purpurea* and *Commiphora myrrha*. RELEEV and Viracea 2,4 were highly active against HIV-1, HIV-2, HSV-1, HSV-2, BVDV and the HCV replicon 122106. Activity against laboratory-derived strains of HIV was detected at greater than 1:30,000 dilutions though lesser levels of activity were found against clinical strains of HIV-1 and HIV-2, suggesting a mode of action involving entry inhibition. MAGI cell-based assays confirmed the ability of the natural product to inhibit HIV entry. Similar levels of activity were detected against HSV-1_{HF} and HSV-2_{MS} in VERO cells, HBV in HepG2.2.15 cells, $BVDV_{NADL}$ in MDBK cells, and against the HCV replicon in Huh-7 cells. Respiratory syncytial virus (RSV) was inhibited, though antiviral activity was not observed against Influenza A or B. Although the mechanism of action of the product against HIV and herpesviruses seems to involve cell surface effects, activity of the product against HBV and in the HCV replicon assay suggests an intracellular mode of action. The range of action of the material also extends to bacteria, where both products were inhibitory in MIC assays to Gram positive and Gram negative bacteria (*S. aureus* and *E. coli*). Thus, the anti-infective attributes render *Echinacea*-derived products amenable to continued development as a treatment for infectious disease. The potent activity against HIV, HSV, and HCV suggests the potential for the development of an effective topical microbicide. A product is being developed for that use. Currently, bioassay-guided fractionation is being performed to define the active molecules responsible for the observed anti-infective activity.

doi:10.1016/j.antiviral.2008.01.075

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Serine Palmitoyltransferase Inhibitor Suppresses HCV Replication in a Mouse Model

Yuichi Hirata [1,*], Takuya Umehara [1], Masayuki Sudoh [2], Fumihiko Yasui [1], Michinori Kohara [1]

[1] Department of Microbiology and Cell Biology, The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan; [2] Kamakura Research Laboratories, Chugai Pharmaceutical Co., Ltd., Kanagawa, Japan

Serine palmitoyltransferase (SPT) is a first-step enzyme in the sphingolipid biosynthetic pathway. NA255 and myriocin is an inhibitor of SPT and suppresses replication of the hepatitis C virus (HCV) replicon. However, it is still unknown whether this SPT inhibitor suppresses HCV replication in vivo. We investigated the anti-HCV effect of SPT inhibitor against intact HCV using chimeric mice with humanized liver infected with HCV genotype 1a or 1b. We administered myriocin into HCV infected chimeric mice and succeeded in reducing the HCV RNA levels in serum and liver to 1/10 to 1/100 of the levels prior to the 8-day treatment. Furthermore, combined treatment with pegylated interferon reduced the HCV RNA levels to less than 1/1000 of the control levels. In conclusion, we elucidated the inhibitory mechanism of HCV replication by SPT inhibitor in vitro and determined that SPT inhibitor inhibits HCV replication in a chimeric mouse model with humanized liver. Our results suggest that SPT may be an effective target of drugs designed to inhibit HCV replication, and that SPT inhibitor has the potential to be a lead compound in the development of new anti-HCV drugs.

doi:10.1016/j.antiviral.2008.01.076

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New Synthetic Histone Deacetylase Inhibitors CGMC0005 and CGMC0006 Effectively Reactivate Latently Infected Human Immunodeficiency Virus Type-1 (HIV-1) from ACH2 and J1.1 CD4+ T Cells

Kee-Jong Hong*, Sung-Soon Kim, Hak Sung Lee, Joo-Shil Lee, Byeong-Sun Choi

Korea Institute of Health, Seoul, Republic of Korea

Histone deacetylase (HDAC) has an important role to induce HIV latently infected cells as HIV reservoir due to the inhibitory function against virus replication by binding HIV-1 LTR promoter. In this study, we treated newly synthesized HDAC inhibitors (CGMC0005 & CGMC0006, Christal Genomics, Seoul, Korea) on the latently HIV-infected cell lines J1.1 and ACH2 to reactivate virus replication from HIV reservoir. In addition, reverse transcriptase inhibitor AZT was treated to the cells to remove viruses excised to cytoplasmor extracellular space for eradicating the latent HIV reservoir. CGMC0005 and CGMC0006 showed better or similar level of safety (CD50: 0.1–0.3 μM) in cytotoxicity compared to SAHA (CD50: 0.3 μM) or PXD-101 (CD50: 0.1–0.3 μM) used as control.

The HIV reactivation test using HIV p24 antigen measurement demonstrated that CGMC0005 treated J1.1 cells and CGMC0006 treated ACH2 cells showed higher reactivation capacity (ED50 $\leq$ 0.1 μM) than SAHA or PXD-101 treated control cells, and other cases showed similar level of reactivation. Cell cycle changes after HIV reservoir reactivation in J1.1 and ACH2 cells were monitored by flow cytometry using propidium iodide. Both of CGMC0005 and CGMC0006 treatments at 0.1 μM elevated S phase cell population while untreated cells were arrested in G1 phase. p24 antigen production was reduced compared to the first reactivation when 0.1 μM HDAC inhibitors were treated again at the sixth day after the first treatment of CGMC0006 with 250 nM AZT. Cell viability was not severely reduced compared to untreated controls. In summary, our research demonstrated that new synthetic HDAC inhibitor, CGMC0005 and CGMC0006, potentiate to break virus reservoir in latently HIV-infected cells selectively. Our findings suggested that both of new inhibitors can be candidates to promote reduction or eradication of the latent HIV reservoir when they treated repeatedly with reverse transcriptase inhibitors like AZT.

doi:10.1016/j.antiviral.2008.01.077

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Optimization of shRNA Features for Targeting Hepatitis C Virus

Brian Johnston [1,*], Alexander Vlassov [3], Brent Korba [2], Kristine Farrar [2], Sampa Mukerjee [2], Attila Seyhan [4], Heini ILves [1], Roger Kaspar [5], Devin Leake [6], Sergei Kazakov [1], Qing Ge [1]

[1] Somagenics Inc., Santa Cruz, USA; [2] Georgetown University Medical Center, Rockville, USA; [3] Ambion, Austin, USA; [4] Open Biosystems, Huntsville, USA; [5] Transderm Inc., Santa Cruz, USA; [6] Thermo Fisher Scientific, Lafayette, USA

Hepatitis C virus (HCV) is a leading cause of liver cirrhosis and hepatocellular carcinoma worldwide. Currently available treatment options are of limited efficacy, and there is an urgent need for development of alternative therapies. We screened in vitro-transcribed, 25-bp short hairpin RNAs (shRNAs) targeting the highly conserved internal ribosome entry site (IRES) of HCV for the ability to silence gene expression. We used a reporter plasmid in which firefly luciferase (fLuc) expression is dependent on the HCV IRES. A 44-nt region of domain IV of the IRES was identified, within which all tested shRNAs efficiently blocked IRES-mediated fLuc expression in transfected human 293 FT cells. Subsequent scans within this "accessible" site with 19 bp shRNAs identified even more potent molecules, providing effective inhibition at concentrations of 0.1 nM. Experiments varying features of the shRNA design showed that, for 25 bp shRNAs, neither the size of the loop (4, 5, 6 or 10 nt) nor the sequence or pairing status of the ends affects activity, whereas in the case of 19-bp shRNAs, larger loops and the presence of a 3′-UU overhang increase efficacy. Comparison of shRNA and siRNA of the same sequence revealed that shRNAs are of similar or greater potency than the corresponding siRNAs in a human hepatocyte cell line chronically infected with HCV subgenomic replicons, and also in mice transfected with the luciferase reporter. The results indicate that shRNAs, delivered as RNA transcripts or chemically synthesized, may be effective agents for the control of HCV.

doi:10.1016/j.antiviral.2008.01.078

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Anti-Influenza A Virus Inhibitory Effect of (−)-Epigallocatechin-3-*O*-Gallate Fatty Acid Monoester Derivatives

Kunihiro Kaihatsu *, Shuichi Mori, Nobuo Kato

Osaka University, Osaka, Japan

Seasonal influenza epidemics and pandemic outbreaks of influenza cause significant disease burdens and mortality in humans. Surprisingly, there are only a few prescribed antiviral drugs for the treatment and prophylaxis of influenza. A neuraminidase inhibitor, oseltamivir phosphate, is the most commonly used antiviral drug, and acts by preventing the release of viral particles from infected cells. However, it has been reported that a highly pathogenic avian influenza (H5N1) possesses resistance to oseltamivir. Moreover, the limited availability of the drug's starting material, shikimic acid, leads to shortages in the drug's supply. Therefore, there is an urgent need to develop a novel anti-influenza virus agent. Here, we describe an approach utilizing Epigallocatechin-3-*O*-gallate (EGCG), a major green tea component, as a novel anti-influenza virus agent. We prepared a series of fatty acid monoesters of EGCG by one-pot lipase-catalyzed transesterification. Our lipase-catalyzed method affords EGCG-monoesters in nearly twice the yield compared to a conventional acid chloride method. Pretreatment of MDCK cells with EGCG-monoesters effectively prevented influenza A/PR8/34 (H1N1) virus infection, and the EC_{50} decreased in an alkyl length-dependent manner. EGCG-monopalmitate exhibited the most potent antiviral activity among the EGCG-monoesters, approximately 24-fold relative to natural EGCG. Further, virus infectivity was drastically reduced when directly incubated with EGCG-monopalmitate. These results suggest that the moderate cell membrane and viral membrane permeability of EGCG-monopalmitate enhanced its accessibility to viral particles and prevented infection at much lower concentrations. Our simple and robust methodology should expand the use of EGCG as a novel antiviral agent. EGCG-monopalmitate is useful for the treatment and prophylaxis of influenza, since it directly interferes with the infectivity of budding viruses. We are studying the antiviral mechanism of EGCG-monoesters to investigate their potential use against oseltamivir-resistant viruses.

doi:10.1016/j.antiviral.2008.01.079