ferent HCV subgenomic (genotype 1b) replicon systems with a 50% effective concentration (EC_{50}) of 1–5 μg/ml. The concentration that reduced the growth of exponentially proliferating Huh 5-2 cells was >30 μg/ml thus resulting in a selectivity index ~25. The anti-HCV activity observed in Huh 5-2 was corroborated by means of RT-qPCR. LCTA-949 inhibited also efficiently the replication of the HCV_{cc} (JFH/J6 chimera) as assessed by RT-qPCR and by monitoring expression of viral antigen. Unlike various selective HCV inhibitors, LCTA-949 is very efficient in clearing cells from HCV replicons. The fact that the compound inhibits subgenomic replicon replication at concentrations that are similar to those that inhibit HCVcc replication, indicate that the compound inhibits intracellular RNA replication. At the ultrastructural level, treatment of either uninfected or infected cells with LCTA-949 results in the formation of multilamellar bodies (MLB). The potential effect of MLB formation on the HCV replication is currently being studied. Semisynthetic hydrophobic derivatives of glycopeptides antibiotics may thus be an interesting route to explore novel antiviral strategies against HCV.

doi:10.1016/j.antiviral.2010.02.394

85

Eradication of Persistent Bovine Viral Diarrhea Virus Infection in Cell Culture by Antiviral Treatment: How to Get Ahead of the Viral Evasion Strategy

Jan Paeshuyse[1,*], Simone Musiu[1], Gerhard Puerstinger[2], Johan Neyts[1]

[1] *Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium;* [2] *University of Innsbruck, Innsbruck, Austria*

The bovine viral diarrhea virus (BVDV) is a member of the family of Flaviviridae. BVDV exists as two biotypes, i.e. cytopathogenic (cp) and non-cytopathogenic (ncp). The ncp variant can establish a persistent infection in live stock as well as in cell culture by eluding the host innate immunity. Here we report on how such a persistent BVDV infection can be completely eradicated from mammalian cells. To this end the persistently infected cells were treated for a number of consecutive passages either with interferon alpha, the interferon inducer polyIC or the small molecule pestivirus inhibitor BPIP [Paeshuyse et al., 2006. J. Virol. 80, 149–160] or a combination thereof. Afterwards the cells were passaged two more times in cell culture medium without inhibitors. For each passage the presence of intracellular and extracellular viral RNA was monitored. An initial experiment resulted in a rapid decline of viral RNA for all inhibitors studied. Combined these data enabled the design of different drug regimes that resulted in the total eradication of BVDV ncp in cell cultures. The results obtained could help better understanding how pestiviruses establish persistent infections and how persistently infected cells can be cleared from such an infection. The latter can be of value for sanitation of precious cell lines that are contaminated by an ncp BVDV infection. Furthermore it is being investigated whether prolonged antiviral treatment of persistently infected cells can restore the innate immunity.

doi:10.1016/j.antiviral.2010.02.395

86

N,N′-Bis(1,2,3-thiadiazol-5-yl)benzene-1,2-diamine Targets the HIV-1 Retroviral Nucleocapsid Zinc Fingers

Christophe Pannecouque[1,*], Beata Szafarowicz[2], Natalia Volkova[3], Vasiliy Bakulev[3], Wim Dehaen[4], Yves Mély[2], Dirk Daelemans[1]

[1] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium;* [2] *Laboratoire de Biophotonique et Pharmacologie, UMR 7213 du CNRS, Université de Strasbourg, Faculté de Pharmacie, 74 route du Rhin, 67401, Illkirch, France;* [3] *Urals State Technical University, Mira Str.19, Ekaterinburg, Russia;* [4] *Chemistry Department, Katholieke Universiteit Leuven, Celestijnenlaan 200F, B-3001 Leuven, Belgium*

From an extensive structure-activity relationship study we have identified *N,N′*-bis(1,2,3-thiadiazol-5-yl)benzene-1,2-diamine (NV038) that efficiently blocks the replication of various strains of HIV-1, HIV-2 and SIV. NV038 inhibited the replication of HIV-1 at a 50% effective concentration of 17.3 μM and was not toxic for the host cells up to 300 μM tested, resulting in a selectivity index greater than 17. The compound was equipotent against several drug resistant virus strains. Time-of-addition experiments indicate that NV038 interferes with an event of the viral replication cycle following the viral entry but preceding or coinciding the early reverse transcription step, pointing towards an interaction with the viral nucleocapsid protein (NCp7). NCp7 is a small protein with two 'CCHC' zinc fingers flanked by basic residues, where both determinants are required for high affinity binding to RNA. The anti-HIV activity of NV038 decreased in the presence of Gag containing VLPs, suggesting its inhibitory effect is caused by an interaction with one of the Gag structural proteins. In fact, *in vitro*, NV038 efficiently chelates the zinc ions and depletes zinc from NCp7, which is paralleled by the inhibition of the NCp7-induced destabilization of cTAR. A chemical model suggests that the two carbonyl oxygens of both esters present in NV038 are involved in the chelation of the Zn^{2+}-ion. Besides the structural features required for zinc chelation other structural elements prove to be crucial for specific target recognition. This new lead and our mechanistic study provide insight into the design of further derivatives against this target with improved efficacy and selectivity.

doi:10.1016/j.antiviral.2010.02.396

87

In Vitro Combination Studies of ANA598 with Anti-HCV Agents Demonstrate Enhanced Anti-viral Activity

Rupal Patel*, Peggy Thompson, Richard Showalter, James Appleman

Anadys Pharmaceuticals, Inc., San Diego, USA

ANA598 is a potent direct-acting antiviral inhibitor of HCV NS5B polymerase that is currently in a Phase II clinical trial in combination with SOC. ANA598 exhibits subnanomolar potency against genotype 1 NS5B polymerase enzymes and dissociates slowly with a $t_{1/2}$ of ~2 h. Nanomolar *in vitro* potency was observed for clinical isolates tested with a mean EC_{50} for genotype 1b of 2.8 nM ($n = 10$) and 27 nM for genotype 1a ($n = 9$). Due to the potential for rapid emergence of resistance mutations to any single direct antiviral used as monotherapy in hepatitis C, future treatment of chronic HCV infection is expected to be in combination with SOC or with complementary direct antivirals. We describe here the *in vitro* assessment of ANA598 in combination with SOC, and several other classes of clinically advanced direct acting antiviral agents.

In vitro combinations of ANA598 with Interferon-α, ANA773 (an oral TLR7 agonist), the HCV NS3/4A protease inhibitor telaprevir, the NS5B polymerase nucleoside inhibitor PSI-6130 (active moiety of R7128), an NS5A inhibitor, and other non-nucleoside inhibitors of NS5B polymerase were conducted in Huh-7 cells containing either the wild type replicon or replicons bearing common palm site mutations (e.g., M414T or G554D). We have previously demonstrated that there is no overlap in viral mutations conferring resistance to NS5B palmsite inhibitors and agents acting at distinct polymerase sites or against the HCV NS3/4A protease *in vitro*. No cytotoxicity was observed for any of the combinations tested. The inhibitory activity of the two agents in combination was compared to the dose response of each agent alone and analyzed assuming Loewe Additivity or Bliss Independence. For each combination evaluated, the antiviral effect between the compounds was determined to be additive to synergistic.

The *in vitro* combination studies (see also Thompson et al. ICAR 2010 abstract) suggest that such combinations may produce a greater viral load reduction and potentially delay the emergence of drug resistance *in vivo*. Collectively, the results provide support for clinical exploration of combination regimens that include ANA598.

doi:10.1016/j.antiviral.2010.02.397

88

Discovery of Novel Small Molecule Inhibitors of Multiple Influenza Strains in Cell Culture

Benjamin Petsch[1,*], Clarence R. Hurt[2], Beverly Freeman[2], Elisabeth Zirdum[1], Anupama Ganesh[2], Alexandra Schörg[1], Anatoliy Kitaygorodskyy[2], Yoko Marwidi[2], Olivier Ducoudret[2], Colm Kelleher[2], William Hansen[2], Vishwanath R. Lingappa[2], Christian Essrich[2], Lothar Stitz[1]

[1] *Friedrich-Loeffler-Institute, Federal Research Institute for Animal Health, Institute of Immunology, Tübingen, Germany;* [2] *Prosetta Bioconformatics, San Francisco, USA*

Efficacy of drugs currently used in anti-Influenza A therapy is decreasing because of emerging viral resistance. Prosetta has discovered novel antiviral compounds, which inhibit the propagation of influenza A virus, by employing a unique moderate throughput screen based upon the interaction of viral proteins with cellular host-factors. Screening of a small molecule chemical library identified 17 distinct chemical series whose activity was validated against Influenza A H7N7, (fowl plague virus (FPV), (Bratislava)) in MDCK cells. In this live virus cell culture assay, six chemical series showed $EC_{50} < 20\ \mu M$. From these early hits, one chemical series was selected for optimization. The synthesis of a small diversity set surrounding this series indicated a robust structure activity relationship existed and the series is currently undergoing optimization for potency, ADMET, and safety profiles. The series has produced multiple compounds with improved anti-viral drug characteristics from the initial screening hit. For example, one compound from this series shows $EC_{50} = 10$ nM, $EC_{99} = 100$ nM with a CC_{50} of 2.5–10 μM. Early pharmacokinetic studies in mice have shown promise, with a mean residence time > 24 h and 31% bioavailability after oral application at 3.6 mg/kg. Compounds in the series have been tested on an Influenza A H1N1 (Puerto Rico 8) strain and they possess activity comparable to that against FPV. Further optimization of potency, ADMET and safety profiles are underway as this series will soon enter animal efficacy studies.

doi:10.1016/j.antiviral.2010.02.398

89

Novel Imino Sugars Potently Inhibit HCV Virion Secretion by Targeting Cellular Endoplasmic Reticulum α-Glucosidases

Xiaowang Qu[1,*], Xiaoben Pan[1], Jessica Weidner[1], wenquan Yu[2], Michael Xu[2,3], Timothy Block[1,2], Ju-Tao Guo[1], Jinhong Chang[1]

[1] *Drexel Institute for Biotechnology and Virology Research, Drexel University College of Medicine, Doylestown, USA;* [2] *Institute for Hepatitis and Virus Research, Hepatitis B Foundation, Doylestown, USA;* [3] *Enantigen Therpeutics, Inc., Doylestown, USA*

Imino sugars, such as deoxynojirimycin (DNJ), are glucose mimetics that competitively inhibit endoplasmic reticulum α-glucosidases I and II, which are essential for glycan processing and folding of viral glycoproteins and are the validated antiviral targets of many enveloped viruses. In our efforts to improve the antiviral efficacy of imino sugars, we discovered recently that *N*-pentyl-(1-hydroxycyclohexyl)-DNJ (OSL-95II) and its derivatives containing modified terminal ring structures, such as PBDNJ0804, demonstrated 25–1000-fold improved antiviral activity against dengue virus than a classical imino sugar NB-DNJ (Chang et al., 2009). In the study reported herein, employing a system for production of infectious HCV particles in cell culture, we found that OSL-95II and PBDNJ0804 also had a superior antiviral activity against HCV with EC_{50} values of 25 and 5 μM, respectively. Consistent with the inhibition of α-glucosidases, both OSL-95II and PBDNJ0804 did not affect the levels of intracellular HCV RNA and non-structural proteins, but efficiently inhibited glycan processing of HCV E2 glycoprotein and induced its degradation. Consequentially, secretion of HCV infectious virions was reduced by 75 and 10,000-fold in the presence of 100 μM of OSL-95II and PBDNJ0804, respectively. Moreover, pharmacokinetics studies showed that the novel imino sugars had good oral bioavailability and were well tolerated by mice and rats in vivo. The superior antiviral efficacy and low likelihood of drug resistant virus emergence hold promise for the novel glucosidase inhibitors to be developed as therapeutic agents against HCV infection, especially as components of combination therapies with IFN or inhibitors of viral proteases and RNA polymerase [Chang, J., Wang, L., Ma, D., Qu, X., Guo, H., Xu, X., Mason, P.M., Bourne, N., Moriarty, R., Gu, B., Guo, J.T., Block, T.M., 2009. Novel imino sugar derivatives demonstrate potent antiviral activity against flaviviruses. Antimicrob. Agents Chemother. 53(4), 1501–1508].

doi:10.1016/j.antiviral.2010.02.399

90

Antiviral Activity of Attachment Inhibitor Against the Pandemic Influenza A (H1N1) Virus

S. Rak[1,*], O. Pyankova[1], A. Chinarev[2], A. Tuzikov[2], N. Bovin[2], A. Agafonov[1], O. Demina[1], A. Ryzhikov[1]

[1] *FSRI SRC VB Vector, Koltsovo, Russia;* [2] *Shemyakin Institute of Bioorganic Chemistry, Moscow, Russia*

The emergence and spread of novel swine-origin influenza A (H1N1) virus in humans highlights the urgent need for new effective therapeutics. An attractive approach for the prevention of influenza infection involves inhibition of virus attachment to susceptible cells by synthetic analogs of cellular receptors. Influenza virus attachment is mediated by the interaction of the viral surface glycoprotein hemagglutinin with host cell surface receptors containing sialooligosaccharides. The goal of this investigation was to study the antiviral effect of low-molecular polyvalent inhibitor