acids from the HR1 central trimeric coiled-coil of the 6HB. To obtain more information on the precise binding mode and mechanism of inhibition of the potent RSV inhibitor TMC353121 (Bonfanti and Roymans, 2009), we determined the high resolution crystal structure of the compound bound at a hydrophobic pocket of the 6HB (Roymans et al., 2009). In contrast to what is generally believed, the binding site of TMC353121 is formed by amino acids from both HR1 and HR2. Binding of TMC353121 stabilizes the interaction of HR1 and HR2 in an alternate conformation of the 6HB, in which direct binding interactions are formed between TMC353121 and both HR1 and HR2. Rather than completely preventing 6HB formation, our data indicate that TMC353121 inhibits fusion by causing a local disturbance of the natural 6HB conformation. If binding with both HR1 and HR2 is a general requirement for the inhibition of 6HB formation by small-molecules, these results may fuel the structure-based discovery of other fusion inhibitors targeting viruses that use class 1 fusion proteins.

References

Bonfanti, J.F., Roymans, D., 2009. Prospects for the development of fusion inhibitors to treat human respiratory syncytial virus infection. Curr. Opin. Drug Discov. Dev. 12, 479–487.

Roymans, D., De Bondt, H.L., Arnoult, E., Geluykens, P., Gevers, T., Van Ginderen, M., Verheyen, N., Kim, H., Willebrords, R., Bonfanti, J.F., Bruinzeel, W., Cummings, M.D., van Vlijmen, H., Andries, K., 2009. Binding of a potent small-molecule inhibitor of six-helix bundle formation requires interactions with both heptad-repeats of the RSV fusion protein. Proc. Natl. Acad. Sci. U.S.A. (December) [Epub ahead of print].

doi:10.1016/j.antiviral.2010.02.353

44

Enterovirus 3C Proteases: Structure-based Discovery of Inhibitors

Rolf Hilgenfeld

University of Luebeck, Luebeck, Germany

Crystal structures have been determined for the 3C proteases of coxsackievirus B3 and enteroviruses 68 and 71 in our laboratory, and for poliovirus, rhinovirus, and hepatitis A virus in other institutions. We are using these structural data for virtual screening and for fragment-based design of non-peptidic inhibitors. Fragment-screening using saturation-difference-transfer (STD) NMR spectroscopy turns out to be particularly successful in identifying small molecules (<300 kDa) that bind to the target. These binding events are confirmed by surface plasmon resonance and X-ray crystallography. Fragments are subsequently linked by medicinal chemistry. An interesting approach to self-ligation of fragments has been developed. An overview of 3C(pro) inhibition, with the latest results included, will be provided.

doi:10.1016/j.antiviral.2010.02.354

45

A Novel 9-Arylpurine Acts as a Selective Inhibitor of *In Vitro* Enterovirus Replication Possibly by Targeting Virus Encapsidation

Hendrik Jan Thibaut[1,*], Lonneke van der Linden[2], Jan Paeshuyse[1], Armando De Palma[1], Leire Aguado[3], Frank Van Kuppeveld[2], María-Jesús Pérez-Pérez[3], Johan Neyts[1]

[1] *Rega Institute for Medical Research, K.U. Leuven, Leuven, Belgium;* [2] *Department of Medical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen Centre for Molecular Life Sciences, Nijmegen, The Netherlands;* [3] *Instituto de Química Médica (CSIC), Madrid, Spain*

We identified a class of 9-arylpurines as selective inhibitors of the replication of various enteroviruses; analogue TP219 [9-(3-acetylphenyl)-6-chloropurine], was selected for further studies. The antiviral activity was assessed by means of CPE and virus yield reduction assays, q-RT-PCR, bioluminescence and antigen detection. TP219 did not inhibit early steps (entry/uptake) nor did it affect polyprotein synthesis/processing or the synthesis of viral RNA, thus suggesting that the drug interacts at a late stage in the replication cycle. Drug-resistant variants were selected that are not cross-resistant to other classes of enterovirus inhibitors (including 3A, 2C and a 3D inhibitor); and they were found to carry several mutations in VP1 and VP3. These mutations were reintroduced in the wild-type genome to confirm their role in the drug-resistant phenotype. The above described experiments revealed that TP219 probably prevents the correct encapsidation of the virion. Mammalian two-hybrid studies are used to explore whether TP219 hinders VP1/VP3 interactions. To study whether the drug prevents virion assembly, we optimized and implemented the nanoLC–MS/MS based SILAC-assays. This allows quantification of capsids and precursors in the infected cells. Since (inhibition of) assembly as such is not a critical step in virus induced cell lysis, probably other (cellular) mechanisms are involved. To study whether specific conformational changes in the capsid inhibit a particular 'death signal' eventually leading to inhibition of virus induced cell death, the effect of TP219 on different apoptosis pathways (in infected and uninfected cell cultures) is being studied. Together, these studies may provide exciting insights in an entirely novel strategy to inhibit picornavirus replication.

doi:10.1016/j.antiviral.2010.02.355

46

Is the Large T Antigen a Target for the Inhibition of SV40 Replication?

Dimitri Topalis*, Ilya Lebeau, Pierre Fiten, Ghislain Opdenakker, Graciela Andrei, Robert Snoeck

Rega Institute for Medical Research, Leuven, Belgium

Background: Simian virus (SV40) belongs to the polyomaviruses, a small DNA virus family that causes severe diseases in immunocompromised patients. The large T antigen (LTag) encoded by SV40 is involved in viral replication and in transformation. The in vitro activity of acyclic nucleoside phosphonates against SV40, especially cidofovir (CDV, HPMPC, Vistide®), has already been shown. But the mechanism of action has not been elucidated yet. Polyomaviruses do not express their own DNA polymerase and require the cellular DNA replication machinery for replication of their DNA.

Methods: CDV resistant SV40 clones were selected for their ability to grow in presence of the drug and their genome sequenced to identify mutations in the LTag gene. In addition, phenotyping of

the different selected clones were performed on BSC-1 cells by viral DNA reduction assay in presence of CDV. The DNA of each clone was extracted and purified for quantification by real time PCR to study the sensitivity of the selected drug resistant clones.

Results: Among the 16 HPMPC-resistant clones selected and genotyped, all bear the mutation V505A localized in the first shell of residues around the ATP binding site of the helicase domain. A212G is present in 14 out of 16 clones, suggesting that this mutation might also be important in the acquisition of drug resistance, or at least in the fitness of the virus. The mutation K697N, present in 6 clones, is localized at the acetylation site of the LTag and interestingly the lysine is mutated into an asparagine which mimics an acetyl-moiety. The presence of this mutation reverses the drug resistance phenotype as measured by quantitative PCR. The other mutations encountered, E92K and R130K described in previous studies and E279G, G649A and C695G which are new mutations as far as we know, may not contribute to the drug resistance phenotype.

Conclusion: The impact of these different characterized mutations has to be investigated in order to understand the mechanism of CDV resistance. Introduction of the mutations A212G, V505A and K697N in SV40 genome by site-directed mutagenesis is ongoing. All these data are consistent with a LTag dependant resistance mechanism.

doi:10.1016/j.antiviral.2010.02.356

Poster Session 1: Retroviruses, Hepatitis Viruses, Respiratory Viruses, Emerging Viruses, and Antiviral Methods

Chairs: 4:00–6:00 pm, Pacific D-O

47

Studies of HIV-1 Integrase Inhibitory Activity of *Wrightia tinctoria*

Periyasamy Selvam[1,*], Kasthuraiah Maddali[2], Yves Pommier[2]

[1] *Devaki Amma Memorial College of Pharmacy, Chelembra, Malapuram, India;* [2] *Laboratory of Molecular Pharmacology, National Cancer Institute, NIH, Bethesda, MD 20892, USA*

Background: The development of antiviral drugs has provided crucial new means to mitigate or relieve the debilitating effects of many viral pathogens. A rich source for the discovery of new HIV-1 infection inhibitors has been and continues to be the 'mining' of the large diversity of compounds already available in nature and exclusively those from botanical extracts. *Wrightia tinctoria* (WT) is used in the Indian system of medicine for the treatment of variety of diseases including HIV/AIDS and enriched with diketo indole derivatives such as indirubin and indigotin, but activity against HIV-1 integrase (IN) not yet been studied. In the present work we studied HIV-1 integrase inhibitory activity of different extracts of *WT*.

Methods: *W. tinctoria* leaf extracts have been studied against inhibition of HIV-1 IN enzymatic activity. All extracts of *WT* were investigated for both 3′-processing (3′-P) and strand transfer (ST) process of HIV-1 IN enzymatic activity.

Results: All extracts exhibited significant inhibitory activity against HIV-1 integrase enzyme (3′-P: 1.9–12 μg/ml and ST: 2.2–12 μg/ml). The aqueous extract (AWT) displayed potent inhibitory activity against both step of HIV-1 IN enzymatic activity (3′-P IC_{50}: 1.9 ± 0.451 μg/ml and ST IC_{50}: 1.4 ± 0.3 μg/ml).

Conclusions: Indole derivatives such as isatin, indirubin, indigotin and tryphanthrin are the principle active constituents of *W. tinctoria*, which may responsible for HIV-1 IN inhibitory activity. The results presented herein substantiate the basis for the discovery of novel natural product IN inhibitors and elucidate the combined usage of medicinal plants in AIDS treatment by Indian traditional practitioners.

Table 1

Extracts	IC_{50} 3′-P, mg/ml	IC_{50} ST, mg/ml
CWT	13.0 ± 3.0	12.0 ± 2.0
MWT	8.7 ± 1.2	4.6 ± 0.4
AWT	1.9 ± 0.5	1.4 ± 0.3
ETWT	2.3 ± 0.4	2.2 ± 0.5
EWT	5.3 ± 0.6	4.6 ± 1.0

doi:10.1016/j.antiviral.2010.02.357

48

Preliminary Evidence of Rapid HBsAg Seroconversion in Patients with Chronic Hepatitis B (CHB) Treated with a DNA-based Amphipathic Polymer

Mamun Al-Mahtab[1,*], Michel Bazinet[2], Andrew Vaillant[2]

[1] *Sheikh Mujib Medical University, Dhaka, Bangladesh;* [2] *REPLICor Inc., Montreal, Canada*

Background: REP 9AC is a DNA-based amphipathic polymer that targets viral glycoproteins important for viral entry and/or release. REP 9AC has been previously shown to result in the rapid clearance of surface antigen and the development of protective immunity in DHBV infected ducks which results in 56% of treated ducks achieving SVR (DHBV DNA negative) at 16 weeks post-treatment. The ability of REP 9AC to treat human patients with CHB is currently being evaluated in a proof of concept trial.

Methods: Patients with CHB were subjected to REP 9AC therapy administered by slow continuous infusion. Safety and virologic response (HBV DNA, HBsAg, anti-HBs) were assessed weekly, either at the trial site or by confirmatory testing (HBsAg, HBeAg, anti-HBs, anti-HBe) of frozen serum samples at a separate location using the Architect™ testing platform.

Results: All patients treated to date have cleared HBsAg and developed protective immunity (anti-HBs) which was observed as early as 7 days following initiation of treatment at higher doses. At the time of abstract submission, one patient has already exhibited clear signs of a sustained virologic response (HBV DNA−, HBsAg−, HBeAg−, anti-HBs+, anti-HBe+) for 12 continuous weeks off treatment after receiving only 23 weeks of treatment with REP 9AC.

Conclusions: These results demonstrate that amphipathic polymers are effective in rapidly reducing HBsAg levels in CHB patients which is a critical event for allowing patients to achieve a rapid seroconversion. Subsequent rapid appearance of anti-HBs and anti-HBe antibodies observed in these patients are the best indicators for achieving SVR and suggest that amphipathic polymers could become an important new tool in the treatment of CHB.

doi:10.1016/j.antiviral.2010.02.358