106

A viable Human Influenza A Virus Lacking Neuraminidase (NA) Activity-isolation and Characterization

Martina Richter[1,*], Sandor Nietzsche[2], Elke Bogner[3], Peter Wutzler[1], Michaela Schmidtke[1]

[1] *Institute of Virology and Antiviral Therapy, Jena University Hospital, Jena, Germany*
[2] *Centre of Electron Microscopy, Jena University Hospital, Jena, Germany*
[3] *Institute of Virology, Charité University Hospital, Berlin, Germany*

NA inhibitor (NAI) genotyping of 25 human influenza A viruses (H3N2) that circulated between 2002 and 2006 in Germany revealed a mixture of full-length and defective NA genes in one of these clinical isolates. In the present study, plaque isolation and purification were applied to get clones of NA deletion mutants and wild-type virus for further characterization. Twenty plaques were randomly picked. Twelve of them were produced by viruses that contained deleted NA genes and 8 by wild-type virus. Three defective as well as three full-length clones were three times plaque purified. Sequence analysis of plaque-purified NA deletion mutants showed that the incomplete gene segment contained 3 deletions and encoded a 25 amino acid protein lacking the coding capacity for the active center of the enzyme. As expected, the defective viruses possessed no neuraminidase activity in a chemiluminescence-based NA assay in contrast to the wild-type virus. Interestingly, the NA-lacking mutant was able to undergo multiple cycles of replication in MDCK cell culture. This was proved by determination of viral titers in the supernatant and by detection of nucleoprotein in infected cells 10, 24, and 48 h p.i. In contrast to the virus expressing full-length NA, the plaque-purified isolates lacking enzymatic activity achieved ~5000-times lower viral yields and virus spread was highly restricted. Differences in virus release were further studied by electron microscopy without and with immunogold labelling. The results from plaque purification as well as RT-PCRs of the NA gene proved that a high amount of NA-deficient viruses was present in the original sample. The full-length NA segment was very faint or not detected by gel electrophoresis whereas a smaller band (~560 bp) was abundantly present. Probably, the shorter defective NA gene segment has a replicative advantage. Wild-type viruses might support the release of mutant viruses from infected cells and act as "helper" virus for spread. Further studies are needed to understand the clinical and therapeutical relevance of these findings.

doi:10.1016/j.antiviral.2011.03.092

107

Favipiravir (T-705) Treatment of Experimental Arenaviral Infection Initiated after the Onset of Clinical Disease

Brian B. Gowen[1,*], Michelle Mendenhall[1], Andrew Russell[1], Donald F. Smee[1], Yousuke Furuta[2]

[1] *Utah State University, Logan, USA*
[2] *Toyama Chemical Company, Ltd., Toyama, Japan*

Lassa and Junín viruses are the most notable of the *Arenaviridae* family of viruses that cause viral hemorrhagic fevers. Ribavirin is the only antiviral drug indicated for the treatment of these severe arenaviral diseases, but has limited efficacy in advanced cases of disease and is associated with toxicity. To further advance preclinical development of T-705 (Favipiravir), a promising inhibitor of arenavirus infection, we have refined and characterized a model of acute arenaviral infection in outbred guinea pigs based on challenge with an adapted strain of Pichindé virus (PICV). Intraperitoneal challenge with 500 plaque-forming units of our guinea pig-adapted passage 19 strain of PICV caused a diffuse infection that was uniformly lethal, associated with fever, weight loss, thrombocytopenia, coagulopathy, and increases in serum aspartate aminotransferase (AST) concentrations. Oral favipiravir treatment (300 mg/kg/day, twice daily for 14 days) reversed disease in sick animals presenting with marked fevers and thrombocytopenia, with all animals fully recovering from PICV-induced disease even when therapy was initiated one week after the infection. Favipiravir effectively reduced viremia and serum AST levels measured during the course of infection. In addition, fever was almost immediately reduced after the initiation of treatment. Limited efficacy was observed when animals were dosed with 150 mg/kg/day or less of favipiravir. The higher dose requirement for favipiravir in the guinea pig PICV infection model compared to the hamster system is not likely due to reduced sensitivity of the p19 guinea-pig adapted strain as it was found to be equally sensitive to the inhibitory effects of favipiravir in cell culture. Further, plasma concentrations achieved following oral favipiravir administration in guinea pigs and hamsters were comparable. We hypothesize that the disparity in effective dosage may be due to less efficient conversion of T-705 to the active triphosphate form or a more rapid systemic elimination in guinea pigs.

doi:10.1016/j.antiviral.2011.03.093

108

Poster Session 2: Hepatitis Viruses, Herpes Viruses, Pox Viruses, Other Antiviral Agents and Medicinal Chemistry *Chairs*: 4:00–6:00 pm Sofia 3 and Kyota

Identification of Alphavirus Inhibitors by Using Virus-Based Assays and a Chikungunya Replicon Cell Line

Tero Ahola[1,*], Leena Pohjala[1,2], Pasi Kaukinen[1], Age Utt[3], Margus Varjak[3], Andres Merits[3], Päivi Tammela[2]

[1] *Institute of Biotechnology, University of Helsinki, Finland*
[2] *Centre for Drug Research, Faculty of Pharmacy, University of Helsinki, Finland*
[3] *Institute of Technology, University of Tartu, Estonia*

We have previously developed luciferase-based methods to screen for inhibitors of Semliki Forest virus (SFV) replication, and discovered novel antiviral nucleosides and betulin-derived compounds, which inhibited both SFV and Sindbis virus (Antiviral Res. 78, 215–222; J. Nat. Prod. 72, 1917–1926). Here, we extend our studies to chikungunya virus (CHKV) by developing a persistent replicon cell line. A screen of 124 natural compounds and 234 clinically approved drugs and other pharmaceutical compounds revealed inhibitors of alphavirus replication in both categories. The hit compounds included 5,7-dihydroxyflavones apigenin and naringenin, coumarins bergapten and coumarin 30 and six drug molecules with 10*H*-phenothiazinyl structure, all showing IC_{50} values against SFV in the low micromolar range. Coumarin 30 and naringenin represented the most potent compounds, with IC_{50} values 0.4 μM and 2.2 μM, respectively, when assayed against a luciferase containing SFV marker strain. The hit compounds also reduced SFV and Sindbis virus induced cytopathic effect and inhibited SFV production in virus yield experiments. A CHKV replicon was constructed containing the virus replicase proteins together with puromycin acetyltransferase, EGFP and *Renilla* luciferase marker genes, and the replicon was transfected into BHK21 cells to yield a stable cell line. A noncytopathic replication phenotype was achieved by combining nsP2 Pro718 to Gly substitution and a five