colorimetric methods (such as the MTS assay), even following prolonged incubation times (>7 days). In order to develop an antiviral screenings-assay against NL63, we explored whether a death-cell protease substrate could be used instead. The substrate used is a quenched peptide (bis-AAF-R110), that releases a fluorophore upon proteolytic-cleavage by proteases the latter released from death cells (Niles et al., Anal Biochem., 2007). After different rounds of optimization the following protocol was developed: Vero118 cells in 96-well plate format were infected with NL63 (MOI = 0.01, 200 μL cell culture, 2×10^4 cells/well, IMDM 5% FBS medium). Cultures were subsequently incubated for 5 days at 35 °C after which 20 μL of the peptide solution (16 μM final concentration) was added. Fluorescence was quantified 2 h after incubation at 37 °C. A roughly 3-fold increase in fluorescence intensity in the infected cultures was observed as compared to the uninfected cultures with a low well-to-well variability. Z′ factors calculated from different experiments were in the range of 0.6–0.8, indicating excellent assay quality. An anti-ACE-II polyclonal antiserum (that prevents coronavirus infection in cell cultures) was used as a positive control and allowed to validate the assay for antiviral screening purposes. In conclusion, in conditions where a viability staining is inadequate to quantify virus-induced CPE, a novel simple and convenient method that detects cell-death and that is suitable for high-throughput screening purposes can be employed.

Acknowledgements: *Funding*: This work was funded by EU FP7 project SILVER (260644).

doi:10.1016/j.antiviral.2011.03.039

54

Effective Prophylactic and Therapeutic Treatment of Yellow Fever Virus with an Adenovirus-vectored Interferon, DEF201, in a Hamster Model

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Many acute arboviral infections are susceptible to interferon (IFN) therapy in various animal models. Yellow fever virus (YFV) is a prime example, where studies in a hamster model of viscerotropic disease have shown utility of IFN treatment. Human YFV disease cases occur annually despite the availability of an effective vaccine. Unfortunately, due to many factors, including cost and toxicity of IFN therapy, difficulty of conducting clinical trials, and lack of monetary incentive, the clinical development of interferon for YFV and other acute arboviral diseases has not been undertaken. To evaluate the efficacy of DEF201, an adenovirus-vectored interferon, a single intranasal (i.n.) dose was administered 4 h prior to YFV challenge of hamsters. A protective effect was observed with a minimal effective dose below 5×10^5 pfu/animal. Treatment was effective when given therapeutically up to 2 days after virus challenge after a single dose of 3×10^7 pfu/animal. At the same dose, DEF201 also displayed prophylactic protection after single dose administration at 7 days prior to virus challenge. We also investigated a novel delivery system, which distributes DEF201 as a fine mist into the nasal cavity. Further studies were performed to evaluate the effect of DEF201 treatment on late-stage immunization. These studies demonstrate the utility and efficacy of interferon produced by DEF201 in the treatment of YFV in a hamster model.

Acknowledgements: Supported by N01-AI-30063 from the Virology Branch, NIAID, NIH.

doi:10.1016/j.antiviral.2011.03.040

55

RNA Interference Mediated Gene Silencing of Influenza A Virus: A Tool for Potent Antiviral Therapy

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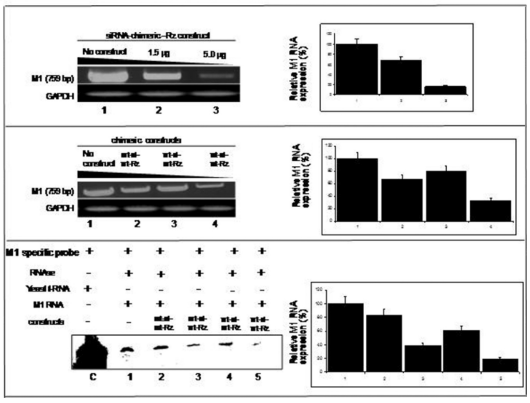
Influenza A virus causes the most prevalent infection of the respiratory tract in humans. Currently available vaccines and antiviral drugs are of limited value because of the rapidly changing antigenic structure of the virus. Clearly, there is a need to develop novel strategies that can potently interfere with the replication of influenza virus .we designed small interfering RNAs against the conserved region of the matrix (M1) and non-structural (NS1) genes of influenza A virus. A decrease in viral gene expression was observed with increasing concentration of siRNAs. We also designed chimeric constructs consisting of siRNA joined by a short intracellular cleavable linker to a known hammerhead ribozyme (Rz) targeted against M1 genome segment of influenza A virus. When this, wt chimeric RNA construct was introduced into a mammalian cell line, along with the M1 substrate, encoding DNA, very significant (67%) intracellular down regulation in the levels of target RNA was, observed. On the contrary, when only the Rz was made, catalytically inactive, keeping the siRNA component unchanged, about 20% reduction in the target M1, specific RNA was observed. This wt chimeric construct showed impressive (>80%) protection against, virus challenge, on the other hand, the selectively disabled mutant constructs were less effective. Therefore, our study demonstrates that the gene silencing technology can provide efficient protection against the influenza virus at desirable levels and may prove as effective antiviral tools.



doi:10.1016/j.antiviral.2011.03.041

56

Antiviral Effect of the Sulfated Polysaccharide, p-KG03, Against Influenza A Virus

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It has been proposed that the sulfated exopolysaccharide, p-KG03, originating from the marine microalgal *Gyrodinium impudicum* strain KG03, inhibits encephalomyocarditis virus (EMCV) replication *in vitro* and stimulates NO production mediated by a