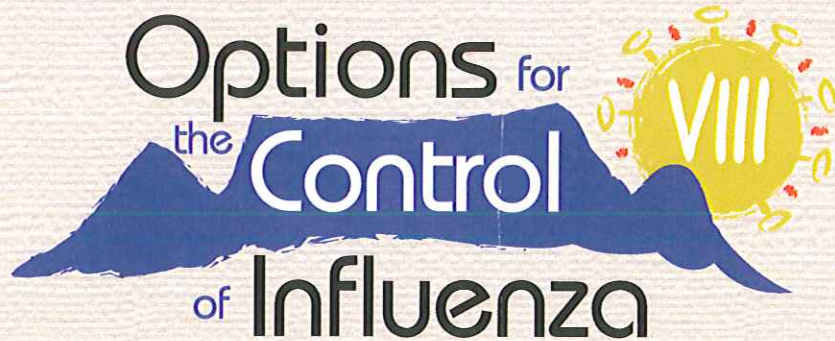


Options for the Control of Influenza



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Abstracts



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presence of T-705. We then examined whether T-705- and placebo-treated viruses from passage 10 would bias the mutation profile toward specific nucleotide mutations. T-705 selection pressure did not change the mutation profile when increasing drug concentrations were applied to T-705-treated variants. Nonetheless, in placebo-treated variants, treatment with T-705 resulted in accumulation of G-to-T and C-to-T transition mutations, which increased linearly with the T-705 concentration, suggesting a dose-dependent effect. Treatment with zanamivir did not significantly increase the number of nucleotide changes and did not bias the natural mutation profile or shift in mutation profile in either T-705- or placebo-treated variants. Conclusions: We have identified an unusual mechanism of viral drug resistance in which drug-selected mutations increase resistance to a mutagen by enhancing polymerase fidelity. This mechanism has not been reported previously in influenza viruses.

O-916

A novel antiviral drug Ingavirin® restores the cellular antiviral response in influenza A virus-infected cells and enhances viral clearance in ferrets

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Background: In order to fight viral infection, mammalian cells are equipped with a rapid antiviral response reaction induced by extra- and intracellular danger signals. This includes activation of PKR, translocation of IRF3 and IRF7 into the nucleus, induction of type I interferon (IFN), and the induction of MxA. As viral RNA effectively induces those factors, most viruses express proteins, which suppress this cellular antiviral response. In this line, influenza A virus codes for non-structural protein NS1. Correspondingly, an influenza virus mutant lacking NS1 is replication defective. Ingavirin® (Imidazolyl Ethanamide Pentandioic Acid) is licensed in Russia for treatment of respiratory infections, because it was shown to target various types of viruses. We hypothesized that the mechanism of action of Ingavirin® is associated with the re-activation of antiviral cellular response pattern, which is usually suppressed by pathogenicity factors such as the influenza A virus NS1 protein. **Materials and Methods:** *In vitro*, A549 cells were infected with influenza A/PR/8/34 virus (wt) at an MOI of 3 in the presence or absence of 1 µg/ml Ingavirin®. We then determined mRNA levels by qPCR and/or protein levels by Western blot or immunocytochemistry, respectively, of the following factors of cellular antiviral response in a time-dependent manner: interferon (IFN) alpha receptor (IFNAR)1, IFNAR2, STAT-1, IRF3, IRF7, MxA, and phosphorylated PKR (pPKR). Expression of type I IFN, interleukin-6, and tumor necrosis factor was determined by ELISA. The replication defective influenza A/PR/8/34 delNS1 virus was used as a positive control. *In vivo*, 3 groups of ferrets (8 animals in a group) were intranasally infected with replicating wild-type influenza virus A/Vienna/1H/2009 (H1N1). Thirty-six hours after the infection, ferrets were orally administered Ingavirin® at a dose of 13 mg/kg or with oseltamivir (Tamiflu) at a dose of 5 mg/kg. Animals in group 3 received the vehicle buffer PBS (controls). Treatment of all animals was repeated in the same manner daily from day 3 (36 hr post-infection) until day 9. Nasal wash samples were collected 2, 4, 6, and 8 days post-infection and evaluated for virus replication by the rapid culture assay (RCA). **Results:** We found that Ingavirin® does not trigger production of IFNs or other pro-inflammatory cytokines in infected cells. However, treatment of wild-type virus-infected A549 cells with Ingavirin® restored and/or enhanced mRNA and protein levels of IFNAR1 and IFNAR2, which were otherwise downregulated by the viral infection. This upregulation of IFN receptors was associated with activation of interferon-dependent molecules such as Stat-1, pPKR, IRF3, and IRF7 and MxA protein, mimicking the molecular pattern of an infection with an NS1 deletion virus. Correspondingly, Ingavirin® significantly accelerated viral clearance from nasal washes of ferrets as compared with the control group on day 4. On day 6, ferrets receiving Ingavirin® had also cleared virus more significantly than the group receiving oseltamivir. No toxic side effects were observed by the addition of Ingavirin®. **Conclusions:** Our results indicate that Ingavirin® should be considered a potent antiviral compound. It appears to effectively restore the naturally evolved innate immune response selectively in virus infected cells. Moreover, this mechanism of action might be a valuable explanation for the proven clinical effectiveness of Ingavirin®.