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Pharmacodynamics of NPI-5291, an Adamantane Class Compound, for Influenza A Viruses

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Influenza A viruses cause yearly epidemics and occassional pandemics. Several antiviral compounds are available for the prevention and treatment of influenza including adamantane derivatives and neuraminidase inhibitors. Although many influenza A viruses circulating in the world today are resistant to the adamantanes, in the future influenza A viruses may again become sensitive to these compounds. We have used dose ranging and dose fractionation studies in our in vitro hollow fiber infection model (HFIM) pharmacodynamic system to determine the optimal dose and schedule of administration of NPI-5291 for the prevention of influenza A virus replication and the supression of the emergence of drug resistant viruses. To perform dose ranging studies in the HFIM system, six hollow fiber (HF) units were charged with 10^2 virus-infected MDCK cells and 10^8 uninfected MDCK cells and continuously infused with different concentrations of NPI-5291 at 37 °C, 5% CO_2 for 4 days. For dose fractionation studies, five HF units were setup with a mixture of 10^2 virus-infected MDCK cells and 10^8 uninfected MDCK cells. One HF unit received no compound, one HF unit received $2XEC_{50}$ dose of NPI-5291 as a continuous infusion and three HF units received a bolus of NPI-5291 designed to deliver a dose equivalent to $2XEC_{50}$ dose on a Q24, Q12, or Q8 schedule followed by a no drug washout with the appropriate half life. Each HF unit was sampled several times to determine the effect of treatment on virus yield by plaque assay. The data show that in the absence of NPI-5291 influenza A virus grew to high titer. In the presence of increasing concentrations of NPI-5291, the yield of released virus decreased. The EC_{50} value of NPI-5291 for this virus was 0.54 μM. The dose fractionation study showed that continuous infusion resulted in superior antiviral effect compared to the fractionated doses. These results show that the pharmacodynamically linked variable is time above the EC_{50} value suggesting that NPI-5291 could be administered on a once daily schedule.

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The Triple Combination of Tenofovir, Emtricitabine and Efavirenz Shows Synergistic Anti-HIV-1 Activity In Vitro

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Background: Tenofovir disoproxil fumarate (TDF), emtricitabine (FTC) and efavirenz (EFV) are the three components of the recently approved once-daily single tablet regimen for HIV-1 infection (Atripla™). To date, the combinatorial effect of these three drugs has not been evaluated for synergetic inhibition of HIV-1. In this study, we tested the dual and triple combinations of tenofovir (TFV, the active antiviral moiety of TDF), FTC and EFV for anti-HIV activity in vitro to determine if the compounds were synergistic.

Methods: The anti-HIV activity of the dual and triple combinations of TFV, FTC and EFV was studied in the human T leukemic MT-2 lymphoblast cell line infected with the wild-type xxLAI strain of HIV-1 using an XTT assay. Antiviral synergy of the dual combinations was evaluated using the MacSynergy™ II software (Prichard and Shipman), by isobologram analysis, and by the median-effect method using the CalcuSyn™ software (Chou and Talalay). The activity of the triple combination was evaluated using the median-effect method. The drug combinations of the two nucleotide analogs TFV diphosphate (TFV-DP) and FTC triphosphate (FTC-TP) and the non-nucleoside reverse transcriptase inhibitor, EFV were also studied at the enzyme level using recombinant wild-type HIV-1 reverse transcriptase (RT) in a standard RT inhibition assay and analyzed by several approaches.

Results: All of the dual drug combinations showed moderate to strong synergistic anti-HIV activity in cell culture. The triple combination of TFV + FTC + EFV also showed synergistic anti-HIV-1 activity in cell culture. At the enzyme level, the combinations of TFV-DP/FTC-TP, TFV-DP/EFV, FTC-TP/EFV and TFV-DP/FTC-TP/EFV were found to be synergistic using median-effect analysis and additive using MacSynergy™ II.

Conclusions: Combinations of TFV, FTC and EFV exhibited moderate to strong synergistic activity against HIV-1 in vitro at both the cellular and RT enzymatic levels. This study furthers our understanding of the mechanism of action for anti-HIV drug interactions and supports usage of the TDF/FTC/EFV combination for the treatment of HIV-1 infection.

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