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Anti-Respiratory Syncytial Virus (RSV) Activity Of 2-5a Antisense Oligonucleotide Chimeras. D.L. Barnard*[1], R.W. Sidwell[1], J.E. Matheson[1], W. Xiao[2], M. Player[2] and P.F. Torrence[2]. [1]Institute for Antiviral Research, Utah State University, Logan, Utah, USA and [2]Section on Biomedical Chemistry, NIDDK, NIH, Bethesda, MD.

A series of composite oligonucleotides with a 2',5'-linked oligoadenylate activator of RNase L covalently linked to deoxyribonucleotides complementary (antisense) to selected RSV RNA sequences were evaluated for inhibition of RSV. Two chimeras, MP207 and MP208, were also modified with a cholesterol or a phosphorothioate group, respectively. Oligonucleotide WX425 inhibited RSV strain A2 at a 50% effective inhibitory dose (EC50) of 5 μM by cytopathic effect reduction assay. The EC50 verified by neutral red uptake assay was 0.3 μM. Ribavirin inhibited RSV A2 at 40 μM. In a virus yield reduction assay, WX425 was a striking inhibitor of RSV replication, with an EC90 = 0.02 μM and a selective index >500. Frequency of compound addition studies showed that adding WX425 twice a day for the first 3 days of the virus infection was a most effective virus inhibitory regimen; a single addition of drug simultaneously with virus was not inhibitory. WX425 was not toxic in stationary cells by neutral red uptake assay or in log phase cells by alamarBlue™ assay at concentrations ≥10 μM. These data suggest that antisense oligonucleotide chimeras should be considered as potential therapeutic agents for RSV infections.

Supported by Contract NO1-AI-35178 from the Virology Branch, NIAID, NIH.

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Effect of Neutralizing Antibody and Interferon on Antibody Resistant Mutants of Poliovirus. M.P. Langford[1], R. Crainic[2] and A.G. Mosser[3]. [1]Department of Ophthalmology, Louisiana State University Medical Center, Shreveport, LA, [2]Unite de Virologie Medicale, Institut Pasteur, Paris, France, and [3]Institute of Molecular Virology, University of Wisconsin, Madison, WI

The antiviral activities of interferon (IFN) and neutralizing antibody act synergistically or additively to inhibit picornavirus production. We investigated this difference by comparing the IFN sensitivities of monoclonal antibody resistant (MAR) mutants of Sabin (Sa) and Mahoney (Ma) strains of poliovirus type 1 (PV-1) in the presence of antigen site specific monoclonal antibodies. The antiviral activity of IFN-α_{2b} against MAR PV-1 Sa mutants and PV-1 Sa or MAR PV-1 Ma mutants and PV-1 Ma were not significantly different. The mean IFN titer of the PV-1 Sa and MAR mutants of PV-1 Sa ($\log_{10}$ 4.0±0.7) were significantly higher than PV-1 Ma and the MAR mutants PV-1 Ma ($\log_{10}$ 2.6±0.5) ($p<0.005$). Addition of site 2 and 3 neutralizing antibody to the overlay medium in PV-1 IFN assays increased the relative IFN titer 0.5-2.0 $\log_{10}$ units. Notably, the relative IFN titer of a PV-1 Ma MAR mutant that had a lysine at position 60 of its VP3 was increased the by 1.3-1.9 $\log_{10}$ by a neutralizing antibody that recognizes the VP3 epitope containing lysine at position 60 in PV-1 Sa antigen site 3. These results support previous work that indicate that neutralizing antibody to antigen site 3 acts with IFN to inhibit PV-1 synergistically.

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Oral Bioavailability Screening of the Antirhinoviral Vinyl Acetylene Benzimidazoles

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The common cold potentially represents one of the largest unmet needs in the healthcare industry today. Lilly has had a program in the area of antivirals and specifically antirhinovirals for many years. The initial program in this area resulted in the discovery and evaluation of two clinical candidates: LY122772 (enviroxime) and LY127123 (enviradene). These compounds suffered both from poor pharmacokinetics in humans and some undesirable toxicology which lead to their abandonment. In light of these results, we have focused not only on optimizing the activity of our compounds but on the oral bioavailability as well. One series which we have recently focused on is the vinyl acetylene benzimidazoles. In particular, the relationship of vinyl acetylene and fluorine substitution was studied. Compounds in this series were found to possess both good activity (enviroxime-like, ~0.05 ug/mL) and promising bioavailability in animals.

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A Novel Anti-Rhinoviral Compound Directed at 3C Protease can Diminish IL-6 and IL-8 Production in a Transformed Bronchial Epithelial Cell Line.

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During a normal rhinoviral infection elevated levels of the pro-inflammatory cytokines interleukin 6 (IL-6) and interleukin 8 (IL-8) are seen in nasal washings. As symptoms decrease the amount of these cytokines return to normal levels. It is thought that if the levels of these cytokines can be brought under control, the symptoms of the infection can be diminished. Using the crystal structure of the rhinovirus 3C protease several potent antiviral compounds directed at human rhinovirus have been synthesized. They are based on the structure of a tripeptide derived from the natural cleavage sequences that fits in the active site of 3C protease. Transformed human bronchial epithelial cells, BEAS-2B cells, can be productively infected by human rhinovirus. These cells produce the cytokines IL-6 and IL-8 as a consequence of infection. When these cells are infected with HRV-14 and given the antiviral compound AG6084 the viral titer of the cell supernatant is diminished compared to infected control cells and the production of cytokines into the media is substantially inhibited. Even when the compound is added 24 hours after infection, the levels of both cytokines can be reduced although IL-6 is diminished more. These findings have implications for the development of anti-rhinoviral agents that will not only block viral replication but also diminish symptoms.