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Rapid In Vivo Development of Influenza A (H3N2) Virus Resistance to Amantadine and Rimantadine. R.W. Sidwell, K.W. Bailey, M.H. Wong, A.G. Morrison, and J.H. Huffman. Inst. for Antiviral Research, Utah State University, Logan, UT 84322-5600 USA.

The development of influenza viruses which are resistant to amantadine and rimantadine has become a serious clinical problem. Studies were run to determine: 1) The rate of development of resistance to these drugs by an H3N2 influenza virus in treated, infected mice. 2) The degree of heterogeneity of resistance to the drugs by individual virus isolates from treated mice. 3) The relative in vivo virulence of the resistant viruses, and 4) the cross-resistance of the viruses. BALB/c mice infected intranasally (i.n.) with influenza A/Port Chalmers/1/73 virus were treated continuously with 1, 0.2, 0.1 or 0.05% amantadine or 1, 0.5, 0.25 or 0.125% rimantadine in the drinking water beginning 2 h (amantadine) or 24 h (rimantadine) prior to virus exposure. In a separate experiment, 50 or 25 mg/kg/day of rimantadine was injected intraperitoneally once daily for 5 days beginning 4 h pre-virus exposure to the infected mice. All treatments significantly inhibited virus-induced arterial oxygen decline as well as lung consolidation and lung virus titers when assayed on day 5. The 1% amantadine and 1% and 0.5% rimantadine doses were lethally toxic to the mice, indicating considerable therapeutic pressure was being applied to the virus infection. Virus recovered after a single passage from the amantadine-treated animals was 1000-fold less sensitive to amantadine as determined in an in vitro cytopathic effect inhibition assay. A 100-fold decrease in response to rimantadine was seen in virus recovered from mice treated with that drug by either route. A cross-resistance to the other drug was seen with all resistant viruses. Individual plaque-purified isolates demonstrated a mixed sensitivity to the drugs, indicating a definite heterogeneity of mutant formation. Resistant viruses recovered from the animals were found to be as virulent in i.n.-exposed mice as non-resistant virus. Studies are underway to determine the specific amino acid substitutions on the M2 protein of the various resistant virus isolates which may confer the resistance seen. [Supported by Contract NOI-AI-15097 from the Antiviral Research Branch, NIAID, NIH].

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Inhibitory Effect of a Series of Protease Inhibitors on Influenza Virus Replication In Vitro.

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A series of protease inhibitors has been evaluated for their inhibitory activity against influenza virus in MDCK cells. Out of 28 synthesized compounds, 14 compounds were inhibitory to the replication of influenza virus at concentrations that were significantly lower than their cytotoxic concentrations. TL-3024 was found to be the most selective inhibitor. Its 50% effective concentration for influenza virus A replication was 3.0 μg/ml, and the selectivity index, which was based on the ratio of the 50 % cytotoxic concentration for the host cell to the 50 % effective concentration for influenza virus A replication, was 55. The corresponding values for ribavirin were 3.6 μg/ml and >56, respectively. TL-3024 was also efficacious in both reducing the viral titer and increasing the survival rate of influenza virus-infected chick embryos. The structure-antiviral activity relationship will be discussed.