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***In Vivo* Influenza-Inhibitory Effects of the Orally Administered Cyclopentane Neuraminidase Inhibitor RWJ-270201.**

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The influenza virus neuraminidase inhibitor RWJ-270201 was evaluated against a spectrum of experimentally induced influenza virus infections in mice. Viruses used were influenza A/NWS/33 (H1N1), A/Bayern/07/95 (H1N1), A/Shangdong/09/93 (H3N2), A/Victoria/3/75 (H3N2), B/Hong Kong/05/72, and B/Lee/40. Treatment was by oral gavage twice daily for 5 days beginning 4 h pre-virus exposure, with doses ranging from 1 to 100 mg/kg/day. Significant efficacy was seen at all doses, as evidenced by prevention of death or lengthening the mean day to death, lessened decline in arterial oxygen saturation, inhibition of lung consolidation, and reduced lung virus titers. Treatment could be started as late as 60 h post-virus exposure, with significant efficacy seen. Increasing viral challenge dose did not affect the antiviral activity seen. In some experiments, comparisons were made to oseltamivir (GS4104), with at least equal antiviral effects seen. Acute treatment with up to 3,000 mg/kg in mice and rats was well tolerated, and rats have displayed no adverse effects at doses up to 1000 mg/kg/day for 5 days. These data indicate RWJ-270201 to be a strong candidate for clinical studies with influenza viruses. [Supported by contract NO1-AI-65291 from the Virology Branch, NIAID, NIH, and a grant from BioCryst Pharmaceuticals and from the RW Johnson Pharmaceutical Research Institute]

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Cross Resistance of Influenza Virus Mutants to NA Inhibitors: Zanamivir, GS4071, and RWJ-270201

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Influenza A and B viruses resistant to NA (neuraminidase) inhibitors, zanamivir and GS4071 (oseltamivir carboxylate), acquire substitutions in the active site at catalytic (152 or 292) or framework residues (119) after prolonged passage in MDCK cells or very uncommonly as a result of treatment in humans. We have assessed sensitivity of the zanamivir- and GS4071-resistant mutants to RWJ-270201, a novel cyclopentane NA inhibitor. The Glu119Ala and the Glu119Gly NA variants were as sensitive to the novel inhibitor as wild type NA. Although the Glu119Gly NA maintained its sensitivity to GS4071, sensitivity of the Glu119Ala and the Glu119Asp NAs to GS4071 was reduced by 3-30-fold. A replacement of Arg at 292 by Lys caused only a moderate (20-fold) reduction in the enzyme sensitivity to RWJ-270201 in contrast to 10,000-fold reduction in sensitivity to GS4071. The Arg152Lys NA variant was resistant to all three inhibitors in NI assays (25-3,000-fold).In addition, we have performed 18-20 passages of influenza A (H1N1 and H3N2) and B viruses in MDCK cells in the presence of RWJ-270201. No change in the NA sensitivity to this inhibitor was detected for influenza B virus. Enzyme activity of the influenza A viruses was significantly reduced as a result of the passage. The molecular basis for this reduction is under investigation at present. Thus, resistance to one or two out of three current NA inhibitors is accompanied by replacement of the framework residues, whereas replacement of catalytic residues leads to resistance, although to different degrees, to all 3 inhibitors. However, substitutions in the NA active site resulted in compromised enzyme function, and their clinical relevance is unclear.

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Age related differences in virus titers in patients in influenza trials of oseltamivir, a novel oral influenza therapy

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Background: The pattern of influenza virus replication varies in different age-groups. Children tend to shed more virus over a longer period of time compared with adults, and it is likely that mild immunosupression in the elderly may also prolong the viral shedding period. This has important implications for influenza transmission, both within families and in residential care. The clinical development of the novel oral antiviral oseltamivir during 1997-9 has studied over 2000 subjects aged 1-96 years in treatment trials, enabling further evaluation of the biology of influenza disease in different populations based on placebo groups. **Methods:** The majority of subjects enrolled into oseltamivir treatment trials gave pre-dose nose/throat swab samples for culture confirmation of influenza infection. Swabs were transported in standard viral transport medium at 4°C to a central laboratory where viral isolation was performed using a standard protocol. Quantitative viral titers were calculated for culture positive baseline samples at a single laboratory in the Netherlands. **Results:** Data from 561 otherwise healthy unvaccinated adults (mean 35y), 100 elderly adults (mean 74y) and 300 children (mean 5.3y) were included in this evaluation. Mean and median baseline virus titers in adults receiving placebo were 1.4 and 3.5 log $TCID_{50}$/mL, respectively. Data for children and elderly patients are currently being analyzed. The effect of age, duration and severity of symptoms and virus titer at baseline for all groups will be presented in full at the meeting. **Conclusion:** Preliminary data analyses suggest that the virus titer and duration of shedding are dependent on the age of the subject, confirming data from previous studies. These findings appear to be consistent through all oseltamivir clinical trials.

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Oral Oseltamivir in Experimental Human Influenza B Virus Infection: Lack of Resistance Emergence

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Oral oseltamivir is a novel neuraminidase (NA) inhibitor effective in the treatment and prevention of human experimental influenza A virus infections. Two randomized, double-blind, placebo-controlled studies were performed to evaluate the activity and safety of oral oseltamivir in the treatment and prophylaxis of human experimental influenza B. For the treatment study, 117 healthy volunteers were inoculated intranasally with 10^7 $TCID_{50}$ of influenza B Yamagata/16/88 virus and after 24h received either placebo (P) (n=39) or oseltamivir (O) 75 mg (n=78) twice daily (bid) for 5 days. For the prophylaxis study, 58 volunteers received either P (n=19), O 75 mg once daily (od) (n=19) or bid (n=20) over 7 days and were inoculated with the virus 24h after first dose. In each case, nasal washes were collected throughout the study period for evaluation of viral titers. Treatment with oral O significantly reduced virus titer AUC compared with P (22.7 vs 131.1 $\log_{10}$ $TCID_{50}$×h/mL; p=0.0023). In addition, the duration of viral shedding was significantly shorter for O (median values: 23.9 vs 95.8 h; p=0.0005) and O treatment was associated with lower symptom scores (median 117 vs 184; p=0.07). When O was used for prophylaxis, serological response was similar in both groups (85% vs 84%). Recovery of virus decreased 24% in the pooled active treatment group compared with P. Median AUC of virus titre was significantly lower in subjects receiving O (10 vs 67 $\log_{10}$ $TCID_{50}$×h/mL; p=0.033), as was duration of viral shedding (36h vs 84h; p=0.034). A total of 112 last day isolates (O: 71, P: 41) were tested for sensitivity by NA inhibition assay to the active oseltamivir metabolite GS4071. None showed reduced sensitivity (range, 75-218 nM) for prophylaxis (mean nM, O: 156, P: 155) or treatment (mean nM, O: 159, P: 156) Oral oseltamivir is an effective inhibitor of influenza B virus replication in experimentally infected humans and does not readily select for influenza B viruses with reduced NA sensitivity.