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Coxsackievirus B3 Mutants Resistant to WIN 63843 Are Attenuated For Virulence In Mice: Molecular Analysis of the Drug Resistant Phenotype. J.M.Groarke,M.A. McKinlay, F.J. Dutko and D.C. Pevear. Sterling Winthrop, Collegeville, PA, USA

WIN 63843 is an antipicornavirus compound primarily targeting infections caused by enteroviruses and rhinoviruses. In order to address the issue of the potential for emergence of drug resistance and the virulence of such viruses in a preclinical model, we chose the Woodruff strain of coxsackievirus B3 (CB3) as the test system. We selected this virus because it has been shown to be very sensitive to this series of WIN compounds, can infect and kill adult Balb/C mice, and the 3D crystal structure is now available. We have isolated two classes of CB3 mutants *in vitro* that are resistant to WIN 63843. The first class, designated LR CB3's, are partially resistant to WIN 63843. Two of these viruses were isolated (LR9 and LR 10) and the VP1 segment of the RNA was sequenced. Both mutants had a single amino acid change at position 1207 which is located in the FMDV loop. LR9 has an Ile to Thr at 1207 and LR10 has an Ile to Met at this position. The mechanism by which these mutations confer partial resistance is not understood. The second class, designated HR CB3's, are completely resistant to WIN 63843. Nine of the ten isolated HR CB3's had a single amino acid change of Ile to Met at position 1092. This position is located in the drug binding pocket of CB3 as inferred from the CB3 crystal structure. The tenth HR CB3 was a double mutant, Ile to Leu at position 1092, and an Ile to Val change at position 1207. In these cases, the mechanism of resistance is most likely due to steric factors which prevent drug binding to the virus. *In vitro* studies with the HR mutants demonstrated that while they were more thermolabile than wild type CB3, they grew as well as the wild type in tissue culture as demonstrated by single cycle growth experiments. In striking contrast, there was a pronounced decrease in virulence *in vivo* for all HR mutants tested. LD_{50} values were 10 fold higher for the mutants and a >2 day delay in the onset of deaths compared to wild type were observed. Of particular interest was the finding that on the day before death, the titers of the virus isolated from the pancreas of mice infected with HR viruses were less thatn 10 pfu/gram compared to the $>10^7$ pfu/gram present in wild type infected tissue. This pronounced reduction in the pancreas and other organs from the HR mice suggests that these deaths were not due to viral induced organ pathology. The cause of death is being investigated.

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Twice daily intranasal GG167 for experimental influenza A. FG Hayden, JJ Treanor, J Esinhart, CU Eason, and EK Hussey. Univ of Va, Charlottesville, VA, Univ of Rochester, NY, and Glaxo, Inc., Research Triangle Park, NC.

GG167, a neuraminidase inhibitor with selective activity against influenza A and B viruses, is active in experimental murine and ferret influenza when administered twice daily (2x/d) to the upper respiratory tract. Placebo-controlled, double blind, randomized studies have evaluated intranasal (IN) GG167 in experimental influenza A/Texas/91 (H1N1) infection in susceptible human subjects. IN GG167 16mg drops 6x/d prevented infection when initiated 4 hours prior to inoculation (INOC) and reduced shedding and limited illness when initiated 26 hours after INOC in an initial study. Three other studies evaluated IN GG167 2x/d. GG167 3.6mg (n=8) or 16mg (n=11) drops beginning prior to INOC prevented shedding ($p<0.005$) and markedly reduced infection rates (shedding and/or rise in HI Ab titer). GG167 one (3.6mg, n=9) or two (7.2mg, n=8) sprays/nostril reduced the infection rate to less than half that observed with placebo and prevented viral shedding in all but 2 volunteers. Compared to placebo (n=15), GG167 16mg drops 2x/d beginning 32 hours post INOC (n=13), reduced the frequency of fever (8% vs 27%), mean sum symptom score (18 vs 37, $p<0.05$), and viral shedding (AUC 0.8 vs 6.9, $p<0.001$). In vitro susceptibility testing of last day isolates from infected subjects is ongoing. In conclusion, IN GG167 2x/d limits experimental human influenza infection.