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**Characterization of an influenza A virus variant selected *in vitro* in the presence of the neuraminidase inhibitor, GS4071**

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Oseltamivir is an effective treatment for influenza. GS4071, its active metabolite, selectively inhibits influenza neuraminidase. Previously it has been reported that the influenza A/Victoria/3/75 (H3N2) variant containing an R292K mutation in the neuraminidase gene exhibits resistance to GS4071. To further understand the potential for the development of resistance against GS4071, we performed *in vitro* drug selection experiments on influenza A/WS/33 (H1N1) containing type 1 neuraminidase. A viral variant with reduced susceptibility to GS4071 was selected by serial passaging the virus in MDCK cells in the presence of increasing concentrations of the inhibitor. Genotypic characterization of the selected virus revealed a single His to Tyr substitution at codon 274 in the neuraminidase gene. No mutation in the hemagglutinin gene has been identified. In an enzyme inhibition assay, the mutant neuraminidase exhibited significantly reduced sensitivity to GS4071, but only minor resistance to zanamivir, another neuraminidase inhibitor. In addition, the mutant enzyme showed two-fold decreased affinity for the fluorogenic substrate 2'-(4-methylumbelliferyl)-α-D-*N*-acetyl-neuraminic acid and two-fold lower enzymatic activity compared to the wild type enzyme. Nevertheless, the viral variant containing the mutant neuraminidase replicated as well as the wild type virus in cell culture. The *in vivo* replication competence of the selected mutant virus is currently under investigation.

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**Studies on the mechanism by which mutations at His274 alter sensitivity of influenza A virus neuraminidase type 1 to GS4071 and zanamivir.**

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GS4071 is a potent and specific inhibitor of influenza neuraminidase (NA). Its binding to NA partly relies on a novel hydrophobic interaction with conserved amino acid residues in the enzyme active site. One of these residues, Glu276, reorients away from the substrate-binding site to form a salt bridge with Arg224 upon GS4071 binding to NA. A viral variant selected *in vitro* for reduced susceptibility to GS4071 contains a mutation His274Tyr that is located in the vicinity of Glu276. To understand the mechanism of drug resistance due to His274Tyr, we expressed in mammalian cells a series of NA variant proteins containing various substitutions at position 274 and studied the drug susceptibility of these mutant enzymes. Replacement of His274 with residues bearing side-chains with larger volumes (Tyr or Phe) reduced the sensitivity to GS4071. In contrast, replacement of His274 with residues bearing side-chains with smaller volumes (Gly, Asn, Ser, or Gln) results in higher or unchanged sensitivity to GS4071. These observations suggest that the loss of sensitivity to GS4071 in the His274Tyr mutant might be due to the loss of the hydrophobic interaction between GS4071 and NA, through blocking the reorientation of Glu276 by the bulky side chain of Tyr at position 274. Previous studies have shown a slow binding component to the inhibition of NA by GS4071 believed to result from the reorientation of Glu276. Loss of this slow-binding inhibition in the His274Tyr mutant NA, but not in His274Asn, suggests that this conformational change cannot occur in the His274Tyr mutant NA upon GS4071 binding. Interestingly, His274Asn displays reduced sensitivity to zanamivir (GG167) and a close analogue in which the guanidino function is replaced by an amine. These data indicate that the volume occupied by the amino acid side-chain at position 274 can influence the sensitivities of influenza NA to both GS4071 and zanamivir.

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Generation and Characterization of a Mutant of Influenza A virus selected with the Neuraminidase Inhibitor RWJ-270201

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RWJ-270201 is a potent and selective inhibitor of influenza neuraminidases. To determine whether viruses exposed to RWJ-270201 may generate resistant variants, A/Singapore/1/57 virus was passaged in the presence of increasing concentrations of the drug. After the 5th and 11th passages of the virus grown in the presence of RWJ-270201, the $IC_{50}$ of the drug against the neuraminidase (NA) enzyme remained unchanged compared to the wild type virus, indicating that there was no change in the active site of the neuraminidase enzyme. The virus was further passaged in the presence of increasing concentrations of the drug (up to 1 mM). After 15 passages with drug (D15), viruses were analyzed using both plaque and neuraminidase assays. Plaque assays performed with the wild type strain of A/Singapore/1/57 in the presence of RWJ-270201 showed good protection with an $IC_{50}$ of less than 1 nM. Conversely, the $IC_{50}$ of RWJ-270201 against the resistant strain (D15) was approximately 300 μM. RWJ-270201 and GG167 showed a 10-20 fold increase in the $IC_{50}$ against the NA enzyme of the D15 resistant strain compared to the wild type strain. However, GS4071 exhibited approximately 5000 fold increase in $IC_{50}$. Sequence analysis of the NA gene of the D15 virus revealed a change in a residue associated with the active site (Arg 292 ->Lys). The same mutation has been reported earlier with both GG167 and GS4071 and the mutant virus has been shown to be less virulent than the wild type. The D15 resistant virus also contained a change in hemagglutinin receptor binding site (Gly 130 -> Asp).

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ANTIINFLUENZA AND ANTI-HSV ACTIVITY OF THE AMINOCAPROIC ACID DERIVATIVE ACEMIN

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Our previous researches showed that inhibitors of proteolysis are perspective antiviral compounds. Ukrainian Ministry of Public Health had permited usage of inhibitor of protelysis E-aminocaproic acid as antiviral on the basis of results of our investigations. The goal of this research was study of antiviral action of acemin which is sodium salt of acetyl-aminocaproic acid. Antiinfluenza activity of acemin in vitro was studied in tissue culture of chorioallantoic membranes of chick embryos. Influenza strains A/Hong Kong/1/68 (H3N2) and A/Odessa/95 (H3N2) were used. The minimal effective doses of acemin were 5.0 mg/mL for virus A/Odessa/95 and 25.0 mg/mL for virus A/Hong Kong/1/68. We also studied action of acemin on HSV-1 (strain US) and HSV-2 (strain VN) replication in primary trypsinized culture of chick embryos cells. The compound demonstrated antiherpetic activity to both strains. Influence of acemin on protection of animals during lethal experimental influenza was studied after intranasal infection of mice with A/PR/8/34 (H1N1) strain. 10% acemin solution was effective when it was injected subcutaneously.