Oral Session VII — Respiratory Virus Infections

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A single sequence change destabilizes the influenza virus neuraminidase tetramer. J. M. Colacino[1], E. Garman[2], N. Y. Chirgadze[1], K. A. Staschke[1], K. G. Murti[3], G. Taylor[4], G. Air[5] and W. G. Laver[6]. [1]Lilly Res. Labs., Indianapolis, USA; [2]Oxford Univ., UK; [3]St. Jude Children's Res. Hosp., Memphis USA; [4]Univ. of Bath, UK; [5]Univ. of Okla. Health Sci. Ctr., Oklahoma City, USA; [6]The John Curtin Sch. of Med. Res., Canberra, Australia

A single change (E119G) in the influenza A virus (N9) neuraminidase (NA) results in resistance of the enzyme to the NA inhibitor 4-Guanidino-Neu5Ac2en (GG167). This change results in the loss of a salt link between E119, which sits in a pocket in the bottom of the active site of the enzyme, and the 4-guanidinium moiety of the inhibitor. NA "heads" of the resistant enzyme have greatly reduced NA activity and have produced only a few small crystals under conditions in which the wild-type enzyme readily formed large crystals. These small crystals were of sufficient quality to yield x-ray crystallographic data which confirmed the E119G change and demonstrated the presence of a strong structural-water molecule in place of the glutamate carboxylate. EM examination of the mutant NA "heads" demonstrated the presence predominantly of monomers with a few tetramers and dimers. The low level of enzymatic activity as well as the small number of crystals obtained were probably from the few tetramers remaining intact in the preparation. The purified wild-type and GG167 resistant enzymes were treated with the homobifunctional NHS-ester cross linker, DTSSP. SDS-PAGE of the treated enzymes revealed cross-linked dimers of the wild-type enzyme whereas only monomers of the DTSSP treated mutant enzyme were observed. An examination of the known x-ray crystallographic structure of the wild-type NA reveals a salt-bridge between E119 and R156 of the same monomer. Since R156 is situated at the interface between monomers, this electrostatic interaction may contribute to the stability of enzyme tetramers. It is suggested that the E119G alteration in the GG167-resistant NA leads to the abrogation of this interaction and thus to the instability of the NA tetramers.

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GS 4071 Is a Potent and Selective Inhibitor of the Growth and Neuraminidase Activity of Influenza A and B Viruses *In Vitro*. D.B. Mendel[1], C.Y. Tai[1], P.A. Escarpe[1], W.-X. Li[1], C.U. Kim[1], M.A. Williams[1], W. Lew[1], L. Zhang[1], N. Bischofberger[1], J.H. Huffman[2], R.W. Sidwell[2], and M.S. Chen[1]. [1]Gilead Sciences, Inc., Foster City, CA, USA and [2]Inst. for Antiviral Research, Utah State Univ., Logan, UT, USA

GS 4071 is a novel, carbocyclic inhibitor of influenza neuraminidases. GS 4104, an orally bioavailable prodrug of GS 4071, is active in animal models of influenza infection. In this study GS 4071 was tested for its ability to inhibit replication of several strains of influenza A and B in tissue culture. GS 4071 was particularly active against H3N2 strains of influenza A, and had activity comparable to or better than the previously described neuraminidase inhibitor 4-guanidino-Neu5Ac2en (GG167) for all strains tested. GS 4071 was also comparable to GG167 in its ability to inhibit influenza neuraminidase in an enzymatic assay, with K_i values of 0.2 nM, 0.5 nM, and 1.2 nM for the neuraminidases of influenza A (H3N2), influenza A (H1N1), and influenza B viruses, respectively. GS 4116, the guanidino analog of GS 4071, was more potent than GS 4071 in tissue culture, and exhibited slow binding to influenza A neuraminidase similar to what has been described for GG167. Both GS 4071 and GS 4116 were selective inhibitors of influenza neuraminidase, showing little or no activity against neuraminidases from human, bacterial, or other viral sources at concentrations up to 1 mM. These data indicate that the oral prodrug of GS 4071 warrants further consideration as a therapeutic for the prevention and treatment of influenza disease. (Supported in part by contract NO1 AI-65291 from the Virology Branch, NIAID, NIH)