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GS 4104 is A Highly Bioavailable Prodrug of the Novel, Potent Influenza Neuraminidase Inhibitor GS 4071. W-X. Li, P.A. Escarpe, M.S. Chen, W. Lew, M. Williams, L. Zhang, C.U. Kim, N. Bischofberger, K.C. Cundy, E.J. Eisenberg, and D.B. Mendel. Gilead Sciences, Inc., Foster City, CA, USA.

GS 4071 is a novel, potent, transition state inhibitor of influenza neuraminidase with activity against both influenza A and B strains *in vitro*. GS 4116, a guanidino derivative of the amino compound GS 4071, is more potent than GS 4071 in enzymatic assays and tissue culture. In this study we determined the oral bioavailability of GS 4071 following a single 10 mg/kg dose of GS 4071 or its ethyl ester prodrug GS 4104, and the oral bioavailability of GS 4116 following a single 10 mg/kg dose of GS 4116 or its ethyl ester prodrug GS 4109 in rats. Both parent compounds and the prodrug GS 4109 showed poor oral bioavailability (2 to 4%) and low peak plasma concentrations (C_{max} = 0.03 - 0.06 µg/mL) comparable to that observed for 4-guanidino-Neu5Ac2en (GG 167). In contrast, GS 4104 was highly bioavailable as parent compound (35%) with no unconverted prodrug detected in plasma. Following oral administration of GS 4104, peak plasma concentrations of GS 4071 (C_{max} = 0.52 µg/mL) were 10- to 1000-fold higher than the IC_{50} values determined for GS 4071 in tissue culture and were maintained for ~6 hours. GS 4104 also exhibited a high degree of oral bioavailability and high, sustained plasma concentrations of GS 4071 in dogs. These data are consistent with the subsequent observation that GS 4104 is orally active in animal models of influenza, and indicate that GS 4104 is a highly bioavailable prodrug for GS 4071 which deserves further consideration as an oral agent for the treatment and prevention of influenza.

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In Vivo Influenza Virus-Inhibitory Activity of an Orally Administered Neuraminidase Inhibitor. R.W. Sidwell[1], J.H. Huffman[1], K.W. Bailey[1], M.H. Wong[1] and C.U. Kim[2]. [1]Inst. for Antiviral Research, Utah State Univ., Logan, UT and [2]Gilead Sciences, Inc., Foster City, CA, USA.

A potent inhibitor of neuraminidase, GS4071, and its prodrug, GS4104, were evaluated against influenza virus infections in mice. The activity was compared with the known neuraminidase inhibitor, 4-guanidino-Neu5Ac2en (GG167) which was evaluated at the same time. Treatment with GS4104 at doses of 10 and 1 mg/kg/day administered by oral gavage (p.o.) twice daily for 5 days beginning 4 h pre-virus exposure was significantly effective in preventing deaths in mice infected with influenza A (H1N1) virus. GS4071 and GG167 were less effective, only the 10 mg/kg/day dose resulting in survivor increases. In another study, p.o. treatment with GS4104 and GG167 significantly inhibited infection induced by influenza A (H1N1) and influenza B viruses, preventing death and significantly reducing virus titers in the lungs. GS4104 was considered more active in inhibiting lung virus titer. A lethal infection of influenza A(H3N2) was also markedly inhibited by GS4104 and GG167, the GS4104 treatment resulting in greater prevention of death, arterial oxygen decline, lung consolidation, and lung virus titers. Delaying p.o. treatment of either compound to begin as late as 60 h post-virus exposure was still inhibitory to a lethal influenza A (H1N1) virus infection. Intranasal instillation with GS4071 was significantly inhibitory to influenza virus infections at doses as low as 0.01 mg/kg/day; GG167 was effective at 0.1 mg/kg/day. No signs of toxicity were seen in any experiment; doses as high as 800 mg/kg/day for 14 days were tolerated in p.o.-treated rats. [Supported by contract NO1 AI-65291 from the Virology Branch, NIAID, NIH, and by a grant from Gilead Sciences, Inc.]

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Efficacy of GS 4104 in Ferrets Infected with Influenza A Virus. C.U. Kim[1], N. Bischofberger[1], M.A. Williams[1], W. Lew[1], J. Merson[2], C. Sweet[3]. [1]Gilead Sciences Inc., 353 Lakeside Dr., Foster City, CA 94404; [2]Pfizer Central Research, Sandwich, Kent CT139 NJ, U.K.; [3]University of Birmingham, Edgebaston, Birmingham, B15 2TT, U.K.

A novel carbocyclic neuraminidase inhibitor, GS 4071 exhibits potent activity against influenza A and B strains with IC_{50} values in the range of 0.7 - 150 nM in tissue culture. GS 4104, the ethyl ester prodrug of GS 4071 showed oral bioavailability of 30 - 100% in three species (mice, rats and dogs). Previously, *in vivo* oral efficacy of GS 4104 was demonstrated in a mouse model of influenza infection. The present study examined the efficacy of orally administered GS 4104 in ferrets infected with influenza A (H3N2) virus In the ferret, bioavailability of GS 4071 after oral administration of GS 4104 (5 mg/kg) was 11%. Oral administration of GS 4104 at 5 mg/kg, bid for 3 days beginning 2 hours post infection, substantially reduced the febrile response. No fever was evident in the group treated with 25 mg/kg of GS 4104. The number of inflammatory cells infiltrating the upper respiratory tract (URT) was reduced approximately 10 fold up to 96 hours. GS 4104 at 25 mg/kg oral dosing showed a significant reduction (about 8 fold) in URT virus titer as measured in nasal lavages during the height of the infection at 30 and 36 hours. No nasal signs (sniffles, discharges, breathing by mouth) were observed in any ferrets treated with 5 mg/kg orally dosed GS 4104. Efficacy demonstrated here against ferret flu symptoms (pyrexia, nasal cell infiltrate, nasal signs) justifies a further evaluation of GS 4104 in human influenza infection.

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Efficacy of the Neuraminidase Inhibitor GG167 in the Treatment of Influenza Virus Infections. F Hayden, Univ of Virginia, Charlottesville, VA; A Osterhaus, Erasmus Univ Hosp, Rotterdam; J Treanor, Univ of Rochester, Rochester, NY; D Fleming, Birmingham UK; F Aoki, Univ of Manitoba, Winnepeg; K Nicholson, Univ of Leicester, Leicester; H Hirst, Glaxo Wellcome, Inc. Research Triangle Park, NC; Oliver Keene, Kevin Wightman, Glaxo Wellcome plc, Greenford, UK

The sialic acid analog GG167 (zanamivir) is a selective inhibitor of influenza A and B virus neuraminidases. When administered directly to the respiratory tract, it has potent antiviral effects in experimental infections of animals and humans. This study assessed the therapeutic activity of GG167 in adults with acute influenza. Two randomized, double-blind, placebo-controlled, multicenter studies were conducted in North America and Europe during the 1994-1995 influenza season. Adults with illness ≤48 hours dura-tion were randomly assigned to one of three treatment groups in a double-dummy fashion: 10 mg GG167 by inhalation, 6.4 mg by intranasal spray and 10 mg by inhalation, or placebo. Treatments were self-administered twice daily for five days. Of 417 enrolled patients, 63% had proven influenza virus infection (56% A, 44% B). For all infected patients, the median time to alleviation of all major influenza symptoms was 1 day shorter in the inhaled (P=0.053) or inhaled/intranasal (P=0.025) GG167 groups compared to placebo (5 days). Among those with febrile illness on enrollment or those treated ≤30 hours from symptom onset, the median time to illness alleviation was 3 days shorter in each of the GG167 groups (P≤0.01) compared to placebo (7 days). Nasal wash viral titers in the inhaled/intranasal GG167 group averaged 2.1 $\log_{10}$ $TCID_{50}$/ml lower on day two and 1.5 $\log_{10}$ lower on day four compared to placebo (P=0.047) Topical GG167 was well tolerated. Direct respiratory tract administration of GG167 is safe and provides symptom benefit if begun early in the course of influenza A or B virus infections in adults.