Preliminary Communication ■

Safety and Efficacy of the Neuraminidase Inhibitor GG167 in Experimental Human Influenza

Frederick G. Hayden, MD; John J. Treanor, MD; Robert F. Betts, MD; Monica Lobo, MD;
James D. Esinhart, PhD; Elizabeth K. Hussey, PharmD

Objective.—The current study evaluated whether intranasal administration of the sialic acid analog 4-guanidino-Neu5Ac2en (GG167), an inhibitor of influenza virus neuraminidase, was effective and safe in either preventing or treating experimental human influenza.

Methods.—Four randomized, double-blind, placebo-controlled trials involving three prophylaxis limbs, two early treatment limbs, and one delayed treatment limb were conducted.

Setting.—Isolation in individual rooms.

Participants.—Susceptible (serum hemagglutination-inhibition antibody titer $\leq$1:8) adult volunteers (n=166) were inoculated intranasally with 10^5 $TCID_{50}$ influenza A/Texas/91 (H1N1) virus.

Intervention.—GG167, 3.6 to 16 mg, was administered intranasally two or six times daily beginning 4 hours before inoculation (prophylaxis) or 1 or 2 days afterward (early or delayed treatment).

Main Outcomes.—Virological measures were frequency of infection based on viral shedding and/or seroconversion (prophylaxis) or quantitative viral shedding based on titers and duration of virus recovery (treatment). Clinical measures were the frequency of febrile illness and symptom severity scores.

Results.—Intranasal GG167 was well tolerated for both prophylaxis and therapy. For all dose groups combined, GG167 prophylaxis was 82% effective in preventing laboratory evidence of infection and 95% effective in preventing febrile illness ($P<.01$ vs placebo). Early treatment with GG167 reduced peak viral titers by 2.0 $\log_{10}$, the median duration of viral shedding by 3 days, and the frequency of febrile illness by 85% ($P<.05$ for each comparison). Other measures of illness were reduced by approximately 50% to 70% in the GG167 dosing groups. Twice daily dosing was as effective as six times daily.

Conclusions.—Direct respiratory administration of the selective neuraminidase inhibitor GG167 appears safe and effective for both prevention and early treatment of experimental influenza. Influenza virus neuraminidase is important for viral replication in humans.

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INFLUENZA neuraminidase (sialidase), one of two major surface glycoproteins of both type A and B viruses, is essential for viral replication in vitro.[1] Cleavage of terminal sialic acid (N-acetylneuraminic acid) residues from cellular and viral glycoconjugates prevents viral aggregation and allows release of virus from infected cells.[1-4] Neuraminidase effects on respiratory tract mucus may facilitate viral penetration to epithelial cells[5] and possibly enhance the pathogenicity of certain strains through modification of the viral hemagglutinin.[6] In animals, immunization with purified neuraminidase or administration of neuraminidase-specific antibody reduces viral replication and modifies disease.[7,8] Neuraminidase-specific antibody levels also appear to correlate with protection against human influenza,[9,10] and neuraminidase vaccines provide partial protection against experimental and natural influenza in humans.[11,12] Therefore, this enzyme represents a suitable target for antiviral chemotherapy.

Two decades ago, analogs of sialic acid[13,14] were shown to inhibit neuraminidase action and influenza replication in cell culture. However, these agents lacked potency and were not active in animals.[15] Characterization of the crystallographic structure of neuraminidase[16,17] and its complex with sialic acid[18] enabled the synthesis of other derivatives. One of these, 4-guanidino-Neu5Ac2en (4-guanidino-2,4-dideoxy-2,3-dehydro-N-acetylneuraminic acid) or GG167, is a potent and highly specific inhibitor of influenza neuraminidase activity and virus replication in vitro.[19-23] Intranasal GG167 has antiviral activity in animal models of influenza.[19,24] No safety problems have been recognized in animal studies or initial human trials.[25] These studies were undertaken to determine if this neuraminidase inhibitor would prove safe and effective in preventing and treating influenza in experimentally infected humans.

METHODS

Volunteers

The participants were healthy, young adults susceptible to the challenge virus (serum hemagglutination-inhibition [HI] antibody titers of $\leq$1:8). Using previously described methods,[26,27] subjects were isolated in individual hotel rooms from 1 day before inoculation until 8 days afterward. Written informed consent was obtained from each participant in a form approved by the institutional review board of the respective institution. Subjects were compensated for participation.

Experimental Design

The participants were inoculated with ~10^5 50% tissue culture infectious doses ($TCID_{50}$) of a safety-tested pool of influenza A/Texas/91 (H1N1) virus (provided by the National Institute of Allergy and Infectious Diseases, Bethesda, Md) by nasal drops (0.25 mL per nostril). Prelimi-

Table 1.—Summary of Trial Design for Testing Intranasal GG167 in Experimental Influenza A Infection*

Trial (Site)	No. of Subjects	Design	Dose Regimen, Onset	Method of Application
1 (University of Virginia)	48	Prophylaxis	16 mg 6 times per d, −4 h	Drops
		Early treatment	16 mg 6 times per d, +26 h	Drops
2 (University of Virginia)	56	Early treatment	16 mg 6 times per d, +26 h	Drops
			16 mg 2 times per d, +32 h	
		Delayed treatment	16 mg 6 times per d, +50 h	Drops
3 (University of Rochester)	31	Prophylaxis	16 mg 6 times or 2 times per d, −4 h	Drops
4 (University of Virginia)	31	Prophylaxis	3.6 mg 2 times per d, −4 h	Drops
			3.6 or 7.2 mg 2 times per d, −4 h	Sprays

*All trials were randomized, double blind, and placebo controlled. To maintain the blind in the second and third trials, subjects received six daily doses with substitution of placebo at appropriate times for those in the twice daily or delayed treatment groups. For six times daily administration, treatments were given every 3 hours while awake.

Table 2.—Demographic Characteristics of the Study Participants*

Characteristic	All GG167 (n=117)	All Placebo (n=49)
Sex, F/M	31/86	10/39
Race, No. (%)		
Black	13 (11)	6 (12)
Hispanic	4 (3)	1 (2)
Asian	12 (10)	3 (6)
White	85 (73)	37 (76)
Other	3 (3)	2 (4)
Age, y		
Median	21	21
Range	18-41	18-33
Tobacco use, No. (%)		
Current user	3 (3)	3 (6)
Former user	25 (21)	4 (8)
Nonuser	89 (76)	42 (86)
Height, cm		
Mean (SD)	176.5 (9.4)	178.3 (9.6)
Range	150-198	158-198
Weight, kg		
Mean (SD)	72.9 (13.7)	75.5 (13.0)
Range	41-114	50-107

*Pairwise comparisons for continuous and discrete data were performed by using rank sum and Cochran-Mantel-Haenszel tests, respectively; all $P>.6$.

nary testing in 18 susceptible adults found that intranasal inoculation of $\sim10^3$, 10^5, and 10^7 $TCID_{50}$ of this virus caused infections in 50%, 75%, and 80%, respectively. The 50% inhibitory concentration of GG167 averaged 0.02 μg/mL for this virus by plaque assay in Madin-Darby canine kidney (MDCK) cells.[21]

Because a limited number of subjects could be studied at one time, four trials were conducted during 6 months (Table 1). All were randomized, double-blind, placebo-controlled trials and followed similar protocol using the same challenge virus. The trials evaluated prophylaxis (dosing 4 hours before viral inoculation), early treatment (dosing 26 or 32 hours after inoculation), or delayed treatment (dosing 50 hours after inoculation). Dosing continued for 4 (treatment) or 5 (prophylaxis) days.

In the first three trials, GG167, 16 mg (Glaxo Research Institute, Research Triangle Park, NC), or a matching placebo of isotonic saline was administered as nasal drops (0.45 mL per nostril). The volunteers remained supine for 2 minutes and performed a series of head-turning maneuvers after each treatment.[28] The same procedure was used for administering the viral inoculum. In the fourth trial, reduced doses of GG167 were used (3.6 or 7.2 mg), and volunteers were randomized to receive study drugs twice daily by drops or by intranasal sprays (0.1 mL per spray) in an upright position as one or two sprays per nostril. The total daily dose of GG167 ranged from 7.2 to 96 mg.

Nasal washings were collected before viral challenge for detecting respiratory viruses by standard techniques and then each morning for 8 days after inoculation for influenza virus isolation in MDCK monolayers. Frozen aliquots from samples that were positive on initial culture were titered in MDCK cells.[26]

To determine the effect of drug carryover in samples on virus recovery, preliminary studies were conducted with simulated and actual nasal washings collected from GG167-treated, uninfected volunteers and into which virus was added. Inhibition of virus recovery by residual GG167 in the sample was avoided by aspiration of the inoculum after the 1-hour absorption period and a single washing of the monolayer before overlaying with maintenance medium (data not shown). Two washings were used in processing samples from these trials. Acute (day before inoculation) and convalescent (3 to 4 weeks later) serum samples were assayed for HI antibody to the challenge virus.

Oral temperatures were measured four times daily. Symptoms were scored by the volunteers twice daily on a four-point scale (absent to severe), and rhinorrhea was measured by nasal mucus weights.[26,27] Routine safety studies (hematology, blood chemistries, urinalysis) and nasal examinations were performed at baseline, during drug administration, and at discharge. Acetaminophen was allowed for fever and discomfort.

Data Analysis

Analyses were performed using SAS software, version 6.07 (Cary, NC). Comparisons of event frequencies between treatment groups were made by Cochran-Mantel-Haenszel test, stratified by trial. Treatment comparisons of other outcomes (viral titers, days of viral shedding, symptom scores, mucus weights) were based on the stratified Wilcoxon rank sum test. All P values were two sided. Efficacy was calculated as [(rate in placebo−rate in GG167)/rate in placebo]×100.

For prophylactic activity, the primary outcomes of interest were the frequencies of infection, viral shedding, and febrile illness. Infection was defined by positive culture for influenza virus on 1 or more postinoculation days or fourfold or greater rise in serum HI antibody titer. Fever was defined by a confirmed oral temperature of 37.8°C (100.0°F) or higher. For each trial, sample sizes were estimated to detect a threefold difference in infection rates (80% power, α=.05).

For therapeutic activity, the primary outcomes were measures of quantitative viral shedding (days of shedding; peak viral titer; the viral titer over time, calculated as an area under the curve [AUC] value using the trapezoidal rule). Sample sizes were estimated to detect a 1.2 $\log_{10}$ $TCID_{50}$/mL difference in viral titer AUC values between GG167 and placebo (80% power, α=.05). Analyses of other outcomes were made for the combined groups to detect differences in illness measures.

RESULTS

Subjects

The four trials had 166 participants, most of whom were young men. The median age was 21 years. Demographic characteristics were comparable in the GG167 and placebo groups (Table 2). Six subjects (one placebo, five GG167) were excluded from efficacy assessments because of preinoculation samples that retrospectively detected shedding of a nonchallenge virus (two) and/or an elevated baseline HI antibody titer greater than 1:8 (five).

Prophylactic Activity

Among placebo recipients, the frequencies of viral shedding (65% to 83%) and infection (70% to 83%) were similar for the three trials. Overall, 70% (23/33) of placebo recipients shed the challenge virus, and 73% (24/33) had laboratory-proven infection (Table 3). For all GG167 recipients, 3% (2/61) shed virus and 13% (8/61) became infected. Thus, the protective efficacy of GG167 was 96% for viral shedding and 82% for infection ($P<.001$ for each comparison).

Those receiving intranasal drops of GG167, regardless of the dose or dosing frequency, had no evidence of viral shedding and marked reductions in the frequencies of infection (Table 3). The protective efficacy of GG167 nasal drops was 100% against viral shedding and 90%

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against infection ($P<.001$). When GG167 was administered by nasal spray, 29% (5/17) became infected (Table 3), and virus shedding was detected in two subjects. The protective efficacy of GG167 intranasal sprays was 83% for virus shedding and 60% for infection ($P<.05$ for each comparison).

Except for one subject receiving six daily doses, GG167 prophylaxis was completely protective against febrile illness (Table 4). The overall efficacy of GG167 was 95% in preventing fever ($P<.001$). Significant reductions were also observed in total symptom scores, nasal mucus weights, frequencies of upper respiratory tract illness and cough, and acetaminophen use (Table 4). Each of these illness measures was reduced 50% to 80% in the GG167 prophylaxis group compared with the placebo group. The daily symptom scores peaked in the placebo group 2 to 3 days after inoculation (Figure 1). In contrast, the GG167 group showed no important change in symptom scores over time.

Therapeutic Activity

To evaluate the therapeutic effects of GG167, only subjects with laboratory evidence of infection were compared. Early treatment with GG167 significantly re-

duced each measure of viral replication compared with placebo across trials (Table 5). The effects observed with twice daily dosing were comparable to those with six times daily dosing (Table 5). For the combined early treatment group, the duration of shedding after starting treatment was reduced by a median of 3 days, the viral titer AUC by 87%, and the peak titer by 99% (average of 2.0 $\log_{10}$) compared with placebo ($P<.001$ for each comparison). Infected placebo recipients experienced a peak in viral titers on days 2 and 3 after inoculation (Figure 2). In contrast, early treatment with GG167 was associated with a rapid titer decline and reduction in peak titers.

Early GG167 treatment also reduced the occurrence of febrile illness with an overall efficacy of 84% (Table 4, $P<.01$). In addition, treatment was associated with approximate 40% to 65% reductions in total symptom scores, frequency of cough, nasal mucus weights, and frequency of acetaminophen use (Table 4). Early treatment with GG167 reduced illness severity as reflected in lower symptom scores beginning on the evening of the second study day (Figure 3).

In the delayed treatment group, peak viral titers were present, and the majority of subjects were already ill at the time treatment was initiated 2 days af-

ter inoculation. Following GG167 administration, viral titers declined promptly (Figure 2), and the subsequent duration of shedding was reduced by a median of 1 day and the viral titer AUC by 75% (Table 5, $P<.05$). No differences in symptom scores or other illness measures (data not shown) were noted compared with placebo, but this analysis was limited by small sample sizes and confounded by a higher illness frequency before starting treatment in the GG167 group compared with placebo. No recrudescence of viral shedding or rebound in viral titers was observed after cessation of GG167 (Figure 2).

Tolerability

No important adverse events or effects on spirometry or electrocardiograms (data not shown) were recognized during GG167 use. The frequencies of local nasal intolerance were similar in the GG167 and placebo groups (Table 6). Mild to moderate increases in transaminases or less often creatine phosphokinase values were observed commonly (Table 6), but these
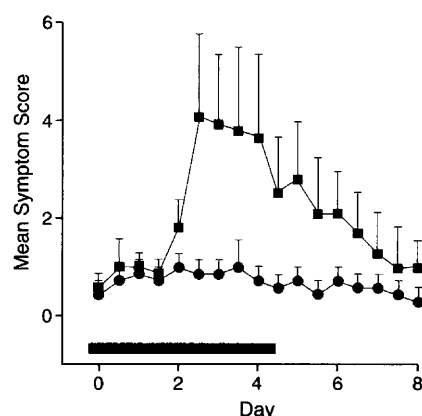

Figure 1.—Effect of prophylactic intranasal GG167 on symptom scores in subjects inoculated experimentally with influenza A/Texas/91 (H1N1). Mean total scores were determined twice daily for the GG167 (circles) (n=61) and placebo groups (squares) (n=33). Vertical lines indicate the upper limit of the 95% confidence interval. The horizontal bar at the bottom of the figure indicates the duration of GG167 administration.

Table 3.—Prevention of Experimental Influenza A/Texas/91 (H1N1) Infection by Intranasal Administration of GG167*

Trial	Dose, Form	Doses per d	No. of Subjects	No. (%) Shedding Virus	No. (%) With Hemagglutination-Inhibition Antibody Rise	No. (%) With Infection
1	16 mg, drops	6	16	0 (0)†	1 (6)†	1 (6)†
3	16 mg, drops	6	10	0 (0)†	1 (10)‡	1 (10)‡
3	16 mg, drops	2	10	0 (0)†	0 (0)†	0 (0)†
4	3.6 mg, drops	2	8	0 (0)‡	1 (13)‡	1 (13)‡
4	3.6 mg, spray	2	9	0 (0)‡	3 (33)	3 (33)
4	7.2 mg, spray	2	8	2 (25)	2 (25)	2 (25)
1, 3, and 4	GG167	Total	61	2 (3)†	8 (13)†	8 (13)†
1, 3, and 4	Placebo	Total	33	23 (70)	23 (70)	24 (73)

*Area under the curve during 8 days after inoculation ($\log_{10}$ TCID$_{50}$×days/milliliters of nasal wash). The frequencies of virus shedding, hemagglutination-inhibition antibody rise, and overall infection were 65%, 65%, and 71%, respectively, in trial 1; 70%, 70%, and 70% in trial 3; and 83%, 83%, and 83% in trial 4.
†$P<.001$ for comparisons between GG167 and corresponding placebo groups.
‡$P<.05$ for comparisons between GG167 and corresponding placebo groups.

Table 4.—Clinical Outcomes in Subjects Receiving Intranasal GG167 for Prophylaxis or Early Treatment of Experimental Influenza A/Texas/91 (H1N1) Infection*

Treatment	No. of Subjects	No. (%) With Fever	No. (%) With URI†	No. (%) With Cough†	Total Symptom Score, Median (Range)†	Nasal Mucus Weight in g, Median (Range)†	No. (%) Using Acetaminophen
Prophylaxis							
GG167	61	1 (2)‡	16 (26)§	7 (11)§	4 (0-71)‡	5.6 (0-65.0)§	5 (8)§
Placebo	33	12 (36)	20 (61)	9 (27)	22 (0-133)	12.0 (0-51.9)	10 (30)
Early treatment (day 1)							
GG167	31	2 (6)§	16 (52)§	5 (16)	12 (0-71)§	7.6 (1.2-22.1)§	4 (13)§
Placebo	26	10 (38)	21 (81)	7 (27)	39 (0-102)	12.5 (1.8-47.0)	10 (38)

*Fever was defined as oral temperature ≥37.8°C (≥100.0°F); upper respiratory tract illness (URI) was defined as two or more respiratory symptoms of any severity on ≥2 days (nasal stuffiness, runny nose, sore throat, sneezing, hoarseness, ear pressure/ache); cough was defined by occurrence ≥2 days.
†Overall days after initiation of GG167 (days 2 to 8 for early treatment/day 1).
‡$P<.001$ for comparisons between GG167 and corresponding placebo groups.
§$P<.05$ for comparisons between GG167 and corresponding placebo groups.

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Table 5.—Virologic Effects of GG167 in the Early or Delayed Treatment of Documented Experimental Influenza A/Texas/91 (H1N1) Infection*

Trial	Dose, Form	Doses per d	Initiation After Challenge	No. of Subjects	No. (%) Shedding Virus	No. (%) With Hemagglutination-Inhibition Antibody Rise	Viral Titer AUC, Mean±SD	Peak Titer (log₁₀TCID₅₀/mL), Mean±SD	Days of Shedding, Median (Range)
Early treatment (day 1)									
1	16 mg, drops	6	26 h	9	8 (89)	5 (56)	1.0±2.2†	1.9±1.5†	1 (0-2)†
2	16 mg, drops	6	26 h	11	8 (73)	11 (100)	1.1±1.4†	1.7±2.0†	0 (0-2)†
2	16 mg, drops	2	32 h	11	5 (45)†	11 (100)	1.0±1.5†	1.0±1.4†	0 (0-2)†
1 and 2	16 mg, drops	Total	Day 1	31	21 (68)†	28 (90)	1.0±1.7‡	1.2±1.7‡	0 (0-2)‡
1 and 2	Placebo	Total	Day 1	26	24 (92)	25 (96)	7.9±6.1	3.7±2.1	3 (0-7)
Delayed treatment (day 2)									
2	16 mg, drops	6	Day 2	12	11 (92)	12 (100)	1.3±1.6†	1.6±1.2	1 (0-3)†
1 and 2	Placebo	Total	Day 2	26	24 (92)	25 (96)	5.2±4.9	3.2±2.1	2 (0-6)

*Overall days after initiation of GG167 (days 2 to 8 for early treatment/day 1; days 3 to 8 for delayed treatment/day 2); area under the curve (AUC) reported as log₁₀ TCID₅₀×day/milliliters of nasal wash. The frequencies of virus shedding, hemagglutination-inhibition antibody rise, and overall infection were 65%, 65%, and 71% respectively, in trial 1 and 87%, 93%, and 93% in trial 2 in the placebo groups.
†$P<.05$ for comparisons between GG167 and corresponding placebo groups.
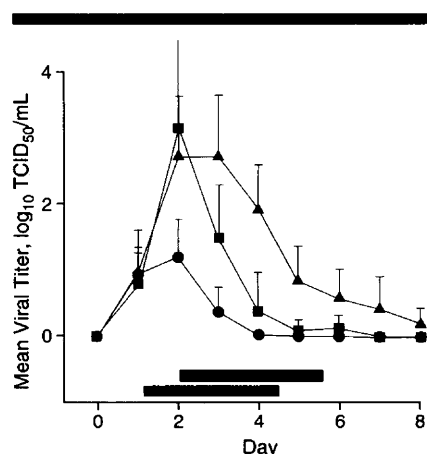‡$P<.001$ for comparisons between GG167 and corresponding placebo groups.

Figure 2.—Effect of early (circles) (n=31) or delayed (squares) (n=12) treatment with intranasal GG167 on nasal wash viral titers following experimental influenza A/Texas/91 (H1N1) infection compared with placebo (triangles) (n=26). The horizontal bars at the bottom of the figure indicate the time of onset and duration of GG167 administration in the early and delayed treatment groups. The mean of the log₁₀ TCID₅₀/mL and upper limit of 95% confidence interval values for infected subjects are shown for each day. The lower limit of assay detectability was 0.5 log₁₀ TCID₅₀/mL. Virus-negative samples were assigned a value of 0 log₁₀ for calculation purposes.

changes did not correlate with GG167 use or presence of influenza infection.

COMMENT

These studies provide the first evidence known to us that an antiviral agent that specifically inhibits viral neuraminidase can be beneficial in human influenza. Intranasal administration of this sialic acid analog was highly effective in preventing experimental influenza infection and illness, when initiated before viral inoculation, and was also effective in inhibiting viral replication and limiting illness in infected persons. Twice daily administration was as effective as six times daily administration, and both were well tolerated in this population.
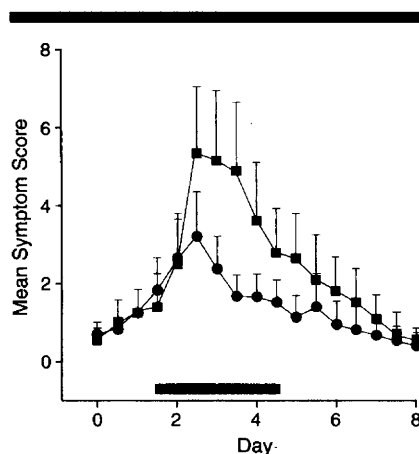
The level of prophylactic efficacy ob-

Figure 3.—Effect of treatment with intranasal GG167 on symptom scores in subjects with experimental influenza A/Texas/36/91 (H1N1) infection. The mean scores for early treatment (circles) (n=31) beginning 1 day after inoculation or placebo (squares) (n=26) groups are shown. Vertical lines indicate the upper limit of the 95% confidence interval. The horizontal bar at the bottom of the figure indicates the time of onset and duration of GG167 administration.

served in these studies compares favorably with that observed in studies of amantadine hydrochloride and rimantadine hydrochloride in experimental human influenza. When these drugs were administered orally beginning 1 to 3 days before viral challenge, the protective efficacy against infection ranged from 7% to 55% (mean of studies, 30%) and against febrile illness from 61% to 100% (mean of studies, 82%) in five representative trials.[27,29-32] In comparison, the combined results with all intranasal GG167 prophylaxis groups indicated that it was 82% effective in preventing infection and 95% effective in preventing febrile illness. Importantly, the results observed with amantadine and rimantadine prophylaxis in experimental influenza accurately predicted the protective efficacy of these drugs against natural influenza.[33]

In experimental animal infections due

to human influenza viruses, intranasal GG167 shows significant effects at low doses given twice daily.[19,24] However, GG167 is essentially inactive after systemic administration despite high blood levels. This finding indicates poor penetration of GG167 into respiratory secretions and is consistent with an extracellular site of action.[24] Intranasal administration was used in the current study because of these observations and the findings that GG167 has poor oral bioavailability and rapid renal elimination in multiple species, including humans.[25] The higher prophylactic efficacy against infection of GG167 given by nasal drops compared with sprays probably relates to differences in intranasal distribution following these different methods of dosing,[34,35] particularly since the virus inoculum was given by drops. In contrast to experimental influenza, naturally acquired influenza commonly involves the lower respiratory tract. In addition to the high frequencies of cough and tracheal irritation observed clinically, direct bronchoscopic evidence and pulmonary function data support this conclusion.[36] Consequently, both intranasal sprays and inhaled aerosols of GG167 are being tested for prevention and treatment of natural influenza in clinical trials.

In contrast to amantadine and rimantadine, GG167 specifically inhibits replication of both influenza A and B viruses.[19,21,22] The strain of influenza A virus used in this study was highly susceptible to GG167 in MDCK cells and in human respiratory epithelial explants.[22] Prior studies have documented a wide range (more than 400-fold) of inhibitory concentrations for human influenza A and B viruses in vitro.[21] However, one relatively resistant virus remained susceptible in vivo,[21] and inhibition of enzymatic activity is observed during a relatively narrow range of concentrations (approximately 10-fold) for the neuraminidases studied to date. This observation is consistent with

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Table 6.—Summary of Adverse Events in GG167 and Placebo Recipients

Adverse Event	GG167 Prophylaxis (n=63)	GG167 Treatment (n=54)	Placebo (n=49)
Recipients with signs or symptoms of nasal irritation, No. (%)			
Total*	9 (14)	8 (15)	6 (12)
Mucosal erosion/ulcer	1 (2)	2 (4)	0 (0)
Mucosal erythema/bleeding	4 (6)	4 (8)	4 (8)
Nasal soreness/irritation	2 (3)	0 (0)	2 (4)
Blood in mucus/epistaxis	3 (5)	2 (4)	1 (2)
Recipients with increases in laboratory measurements, No. (%) [median increase]†			
Alanine aminotransferase	12 (19) [twofold]	1 (2) [threefold]	7 (14) [twofold]
Aspartate aminotransferase	13 (21) [threefold]	4 (7) [twofold]	7 (14) [fourfold]
Creatine phosphokinase	1 (2) [sevenfold]	2 (4) [25-fold]	1 (2) [134-fold]

*The total of individual categories may exceed the overall percentage because multiple signs or symptoms could be present in given subject.

†Changes from within normal range at baseline to above upper limit of normal by day 8. The median increase in those with elevations is listed.

the finding that the active enzyme site is lined by highly conserved amino acid residues. The clinical significance of differences in in vitro susceptibility remains to be determined.

The marked reduction in infection frequency observed with prophylactic use of intranasal GG167 may relate to the postulated role of influenza neuraminidase in facilitating virus movement through respiratory mucus.[5,37] Inhibition of neuraminidase may have prevented virus from reaching respiratory epithelium and initiating infection. In addition, inhibiting subsequent rounds of replication may have reduced antigen loads and prevented detectable humoral immune responses. Although a neuraminidase-deficient variant is capable of low-level replication in mice,[38] our results suggest that influenza neuraminidase is essential for sustained viral replication in humans.

The encouraging results observed in these studies provide the impetus for comprehensive testing of the prophylactic and therapeutic potential of GG167 in naturally occurring influenza and for searching for other antiviral agents directed against influenza virus neuraminidase.

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