Use of the Oral Neuraminidase Inhibitor Oseltamivir in Experimental Human Influenza
Randomized Controlled Trials for Prevention and Treatment

Frederick G. Hayden, MD

John J. Treanor, MD

R. Scott Fritz, PhD

Monica Lobo, MD

Robert F. Betts, MD

Madeline Miller, DVM

Nelson Kinnersley, MSc

Roger G. Mills, MD

Penelope Ward, MD

Stephen E. Straus, MD

A CONTINUING NEED EXISTS FOR antiviral agents against influenza A and B virus infections for treatment of influenza and as a supplementation to vaccines for prevention. The influenza virus neuraminidase is 1 of 2 major surface glycoproteins of influenza A and B viruses. It cleaves terminal sialic acid (*N*-acetylneuraminic acid) residues from cellular and viral glycoconjugates and is essential for sustained viral replication in vitro[1] and probably also in humans.[2] Inhibition of neuraminidase enzymatic action by antibody, mutation, or chemicals causes virus particles to aggregate at the cell surface and with each other. In addition, neuraminidase prevents inactivation of influenza virus by respiratory mucus and likely facilitates infection of the airway mucosa.[3] The enzyme active site is highly conserved across influenza A and B viruses,[4-6] and several novel antiviral compounds have been designed based on the neuraminidase crystallographic structure.[5,7]

Context Influenza virus neuraminidase is thought to be essential for virus replication in humans; however, to date, available neuraminidase inhibitors are limited to zanamivir, which is topically administered.

Objective To determine the safety, tolerability, and antiviral activity of oral neuraminidase inhibitor oseltamivir (GS4104/Ro64-0796) for prevention and the early treatment of influenza in experimentally infected humans.

Design Two randomized, double-blind, placebo-controlled trials conducted between June and July 1997.

Setting Individual hotel rooms; 2 large US university medical schools.

Participants A total of 117 healthy adult volunteers (aged 18-40 years; median age, 21 years) who were susceptible (hemagglutination-inhibition antibody titer ≤1:8).

Interventions All subjects were inoculated intranasally with influenza A/Texas/36/91 (H1N1) virus. For the prophylaxis study, oral oseltamivir (100 mg once daily [n = 12], 100 mg twice daily [n = 12], or matching placebo [n = 13], starting 26 hours before virus inoculation) was administered. For the treatment study, the same drug was given (20 mg, 100 mg, or 200 mg twice daily, 200 mg once daily, or matching placebo [n = 16], in each group starting 28 hours after inoculation). All regimens were continued for 5 days.

Main Outcome Measures Comparing placebo groups with pooled treatment groups, for prophylaxis, outcomes included frequency of infection and viral shedding; for treatment, viral shedding in titers.

Results In the prophylaxis study, 8 (67%) of 12 placebo and 8 (38%) of 21 oseltamivir recipients became infected ($P = .16$; efficacy, 61%); 6 (50%) placebo compared with 0 oseltamivir recipients shed virus ($P < .001$; efficacy, 100%), and 33% of placebo but no oseltamivir recipient had infection-related respiratory illness ($P < .01$). Among infected subjects in the treatment study (n = 69), the viral titer area under the curve of the combined oseltamivir groups (n = 56) was lower (median [interquartile range {IQR}], 80 [23-151] vs 273 [79-306] $\log_{10}$ tissue culture-infective doses$_{50}$ per milliliter × hour; $P = .02$) than the placebo group (n = 13), and the median (IQR) duration of viral shedding with therapy was reduced from 107 (83-131) to 58 (35-59) hours ($P = .003$). Oseltamivir treatment also reduced symptom scores (median [IQR] score-hours, 225 [97-349] vs 400 [189-645]; $P = .05$), and nasal proinflammatory cytokine levels. Transient mild to moderate nausea after dosing was observed in 15 (17%) of 88 oseltamivir and 2 (7%) of 29 placebo recipients (95% confidence interval for difference, −11% to 68%), which was largely prevented by ingestion with food.

Conclusions In these trials, prophylaxis and early treatment with oral oseltamivir were both associated with significant antiviral and clinical effects in experimental human influenza.

JAMA. 1999;282:1240-1246   www.jama.com

The neuraminidase inhibitor zanamivir (or GG167) has been shown previously to have anti-influenza activity in experimentally infected animals[7-9] and humans.[2] Inhaled zanamivir provides clinical benefit in adults with acute, uncomplicated influenza.[10,11] However, the low oral bioavailability, small volume of distribution, and rapid renal elimination of zanamivir[12] has limited its administration to topical (intranasal and/or inhaled) routes of delivery in human studies. These topical routes are not easily used by many patients, including persons who are infirm and young children, and distribution of the drug is not uniform throughout the respiratory tract.[13] Effective delivery of the drug to both upper and lower respiratory tracts may be important for reducing complications and viral transmission. Consequently, considerable interest exists in developing oral inhibitors of influenza neuraminidase.

A carbocyclic transition state analog of sialic acid cleavage, GS4071/Ro64-0802 (3R,4R,5S-4-acetamido-5-amino-3-[1-ethylpropoxy]-1-cyclohexene-1-carboxylic acid) is comparable with zanamivir in potency and selectivity under in vitro conditions.[14-16] Although GS4071 is poorly absorbed, oral administration of the ethyl ester prodrug oseltamivir phosphate (GS4104/Ro64-0796) gives good bioavailablity of GS4071 in multiple species.[17] Oral oseltamivir is active in murine and ferret models of influenza.[18,19] In the rat, oral oseltamivir provides prolonged levels of the parent compound GS4071 in lung tissue and bronchoalveolar lavages.[20] In humans, oral oseltamivir is associated with dose-proportional increases in plasma GS4071 concentrations and a prolonged plasma GS4071 half-life (7-9 hours), which allows for infrequent dosing.[21] The objectives of our study were to determine the safety, tolerability, and antiviral activity of oral oseltamivir for the prevention and early treatment of influenza in experimentally infected volunteers.

METHODS
Volunteers and Infection

The subjects were healthy adults aged 18 to 40 years who were susceptible to the challenge virus on the basis of having serum hemagglutination-inhibition antibody titers with a dilution of 1: 8 or less. Those with concurrent medication use or illness within 1 week were excluded. Only nonsmokers or those smoking fewer than 10 cigarettes daily who agreed to abstain during the study were included. Written informed consent was obtained from each participant in a form approved by the institutional review boards of the University of Virginia, Charlottesville, and the University of Rochester, Rochester, NY, and subjects were compensated for participation.

Using previously described methods,[2,22,23] the subjects were isolated in individual hotel rooms 1 day before inoculation until 8 days after inoculation. They were inoculated by intranasal drops (0.25 mL per nostril) with approximately 10^6 median tissue culture-infectious doses (tissue culture-infective doses$_{50}$) of a safety-tested pool of influenza A/Texas/36/91(H1N1) virus (provided by the National Institute of Allergy and Infectious Diseases, Bethesda, Md). This virus was readily inhibited by GS4071 in Madin Darby canine kidney cells (50% plaque inhibitory concentration <0.1 µg/mL) and in explants of human respiratory epithelium (90% yield inhibitory concentration <0.01 µg/mL) in vitro.[16]

Study Design

Two randomized, double-blind, placebo-controlled, dose-ranging trials were conducted in parallel to test the prophylactic and early therapeutic activity of oral oseltamivir capsules. The placebo capsules were identical in appearance. In the prophylaxis study (University of Rochester), individual volunteers were assigned to 1 of 3 treatment groups: 100 mg once daily (n = 12), 100 mg twice daily (n = 12), or placebo (n = 13). Administration began 26 hours prior to viral inoculation and continued for 5 days. In the treatment study (University of Virginia), the volunteers were assigned to 1 of 5 treatment groups (n = 16 per group): 20, 100, or 200 mg twice daily, 200 mg once daily, or placebo. Admin-

istration began at 28 hours after inoculation and continued for 5 days. Because of the large numbers of subjects in the treatment study, it was conducted in 2 sessions separated by 1 week. Drug was administered under direct observation of the study nurses to ensure compliance. Computer-generated code was used to randomize subjects. The code remained unbroken until all results had been collected and entered into the final database.

Nasal washings were collected before viral inoculation for detecting respiratory viruses by standard techniques and then each morning for influenza virus isolation in freshly inoculated Madin Darby canine kidney monolayers. Washings were also collected each evening on days 2 and 3 after challenge in the treatment study. Frozen aliquots from samples that were positive on initial isolation were subsequently titered in Madin Darby canine kidney cells. Prechallenge and convalescent (3-4 weeks after inoculation) serum samples were tested for hemagglutination-inhibition antibodies to the challenge virus.

Nasal washings collected before viral inoculation and on days 2, 4, and 8 after inoculation were processed for cytokine determinations as previously described.[24] Cytokine levels were determined using commercially available enzyme-linked immunosorbent assay kits and the manufacturer's protocols. The kits were obtained from the following sources: interleukin (IL) 5, IL-6, interferon alfa (IFN-α), and IFN-γ (Endogen Inc, Cambridge, Mass); tumor necrosis factor alpha (TNF-α) (R&D Systems Inc, Minneapolis, Minn). The limits of sensitivity of these assays, as supplied by the manufacturers, were as follows: IL-5 (<2 pg/mL), IL-6 (<1 pg/mL), IFN-α (<3 pg/mL), IFN-γ (<2 pg/mL), and TNF-α (<0.18 pg/mL).

Oral temperatures were recorded 4 times daily, and 14 symptoms (nasal stuffiness, runny nose, sore throat, sneezing, cough, breathing difficulty, muscle aches, fatigue, headache, earache/pressure, feverishness, hoarseness, chest discomfort, overall discomfort) were scored by the subjects twice daily on a

4-point scale (absent to severe). Rhinorrhea was assessed by measuring nasal mucus weights; middle-ear pressure abnormalities were assessed by digital tympanometry completed once daily.[23] Routine safety laboratory studies (complete blood cell counts, differential leukocyte count, platelets, urinalysis, serum electrolytes, calcium, phosphorus, urea, creatinine, glucose, total protein, albumin, amylase, uric acid, triglycerides, cholesterol, aspartate aminotransferase, alanine transaminase, glutamic-oxaloacetic transaminase, alkaline phosphatase, bilirubin, creatine phosphokinase, and lactate dehydrogenase) were performed at baseline and following completion of drug dosing. Acetaminophen was allowed for fever and discomfort.

Outcomes

Infection was defined by a positive culture for influenza virus on 1 or more postinoculation days and/or 4-fold or greater increase in serum hemagglutination-inhibition antibody titer. Upper respiratory tract illness was defined as 2 or more respiratory symptoms (nasal stuffiness, runny nose, sore throat, sneezing, hoarseness, and ear-

ache) of moderate or severe intensity occurring on 1 or more days after challenge. Lower respiratory tract illness was defined by the presence of cough of moderate or severe intensity on 1 or more days after challenge. Fever was defined by a confirmed oral temperature of 37.8°C or higher.

In the prophylaxis study, the primary outcomes of interest were the frequencies of viral shedding and infection. The secondary outcomes were the symptom scores over time and the frequencies of infection-associated fever and upper respiratory tract illness. Sample sizes were based on prior findings with this virus and zanamivir administration.[2] With 14 subjects per group, a comparison of placebo vs the pooled active treatments had an 80% power to detect a significant difference (2-sided, 5% level) between infection or virus shedding rates of 80% in those taking the placebo vs 30% taking active drug.

In the treatment study, the primary outcome measure was the quantity of virus shed over time in nasal washings. This was calculated as an area under the curve (AUC) for viral titers over the 7 days after treatment initiation. The secondary efficacy end points included the

time to cessation of viral shedding, total symptom score AUC, nasal mucus weights, frequencies of upper respiratory tract illness, cough, fever, middle ear pressure abnormalities, and concentrations of cytokines in nasal lavage fluids. The time to alleviation of illness was defined as the time from the beginning of the study treatment to the time that 7 key symptoms typical of natural influenza had reduced to absent or mild. Similarly, the time to cessation of viral shedding was defined from the beginning of the study treatment. Sample sizes were based on prior findings with this virus and zanamivir treatment.[2] With 16 subjects per group, a comparison of placebo vs pooled oseltamivir groups had 80% power to detect a significant difference (2-sided, 5% level) of 4.0 $\log_{10}$ tissue culture-infective doses$_{50}$ per milliliter $\times$ days in the viral titer AUCs (SD, 5.5 $\log_{10}$ tissue culture-infective doses$_{50}$ per milliliter $\times$ days).

Data Analysis

A 2-sided Fisher exact test was used for comparison of infection rates between placebo and the pooled oseltamivir groups and a Wilcoxon rank-sum test was used to compare differences in viral titer AUC values. The time to cessation of viral shedding and the time to alleviation of symptoms were compared using a generalized Gehan-Wilcoxon test. To compare the percentage of subjects in the placebo group and active dose groups exhibiting gastrointestinal tract events during the dosing period, exact 95% confidence intervals were calculated according to the method of Santer and Snell.[25] Sample size calculations were performed using statistical software (PASS 6.0, NCSS, Kaysville, Utah).

RESULTS
Volunteers

The 2 trials enrolled a total of 117 participants, most of whom were young (median age, 21 years). There were no dropouts and all subjects completed the full course of drug administration. In the prophylaxis study, 4 subjects (3 oseltamivir, 1 placebo) were excluded from efficacy analysis because of baseline hem-

Figure 1. Prophylaxis and Treatment Trials
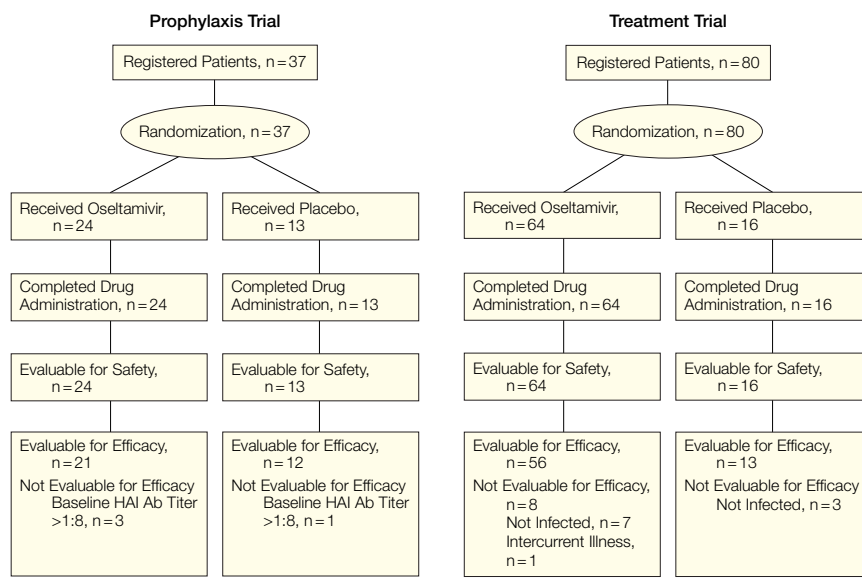
HAI Ab indicates hemagglutination inhibition antibody.

agglutination-inhibition antibody titers with a dilution of more than 1: 8. In the treatment study, 11 subjects (8 oseltamivir, 3 placebo) were excluded because of lack of documented infection or of intercurrent viral illness (1 oseltamivir) (**FIGURE 1**). All volunteers were included in the analysis of safety.

Prophylactic Activity

Among the 12 evaluable placebo recipients, 6 (50%) had recovery of the challenge virus from nasal washings and 8 (67%) had laboratory-confirmed infection. In contrast, none of the 21 evaluable oseltamivir recipients had virus isolated ($P<.001$; 100% efficacy) and only 8 (38%), 4 in each dose group, had serologic evidence of infection ($P = .16$; 61% efficacy). This antiviral effect was associated with reductions in illness measures. Symptom scores over time did not change significantly after viral inoculation in the oseltamivir group, in contrast to the expected peak 2 days after challenge in the placebo group (**FIGURE 2**). Infection-associated upper respiratory tract infection developed in 4 subjects (33%) taking placebo but in none of the subjects taking oseltamivir ($P = .01$). Fever was observed in 3 (25%) and cough in 2 (16%) placebo recipients. No important differences were observed between the once and twice daily oseltamivir dose groups.

Therapeutic Activity

Only the 69 subjects (56 oseltamivir, 13 placebo) with laboratory-documented infection were included in the analysis of therapeutic efficacy. Drug administration was initiated at a time when viral titers were increasing (**FIGURE 3**). Peak viral titers were observed about 1½ days after challenge (12 hours after starting study drug) in both groups and tended to be lower in the oseltamivir recipients. At 24 and 36 hours after initiating study drugs, the median viral titers were reduced by 2.1 log_{10} and 3.5 log_{10}, respectively, in the combined oseltamivir group compared with placebo ($P = .02$ by AUC analysis). Correspondingly, the me-

dian time to cessation of viral shedding was reduced from 107 hours in the placebo group to 58 hours in the combined oseltamivir group ($P = .003$). No significant differences in these virological measures were observed across the oseltamivir dose groups, although the

200-mg once daily group had a higher viral titer AUC value compared with the twice daily dose groups (**TABLE**).

This antiviral effect was associated with significant reductions in illness burden and biochemical markers of host inflammatory responses in the res-

Figure 2. Effect of Oral Oseltamivir Prophylaxis on Illness Following Experimental Influenza A/Texas/36/91(H1N1) Inoculation
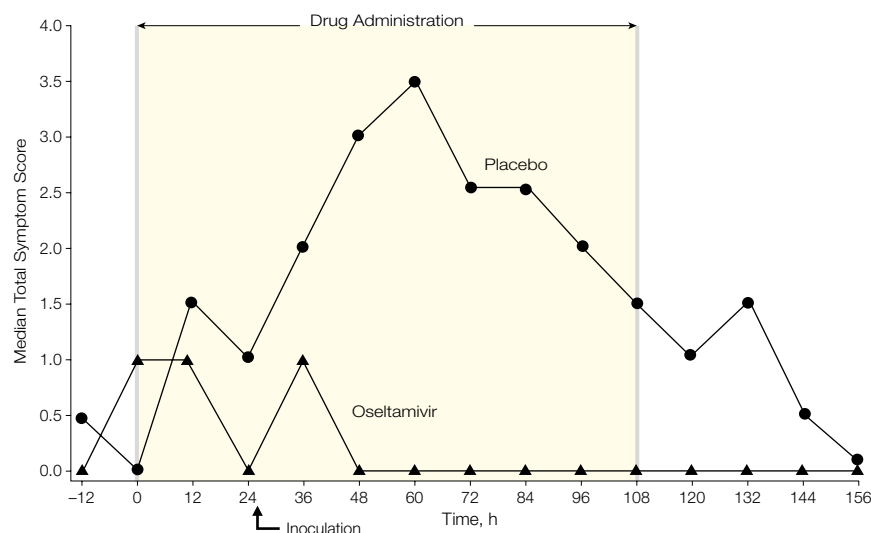
The total symptom score area under the curve value was lower in the combined oseltamivir groups (n = 21) compared with placebo (n = 12); $P = .02$. Fourteen symptoms related to influenza were included in the score.

Figure 3. Effect of Oral Oseltamivir Treatment on Vital Titers in Nasal Lavages Following Experimental Influenza A/Texas/36/91(H1N1) Infection
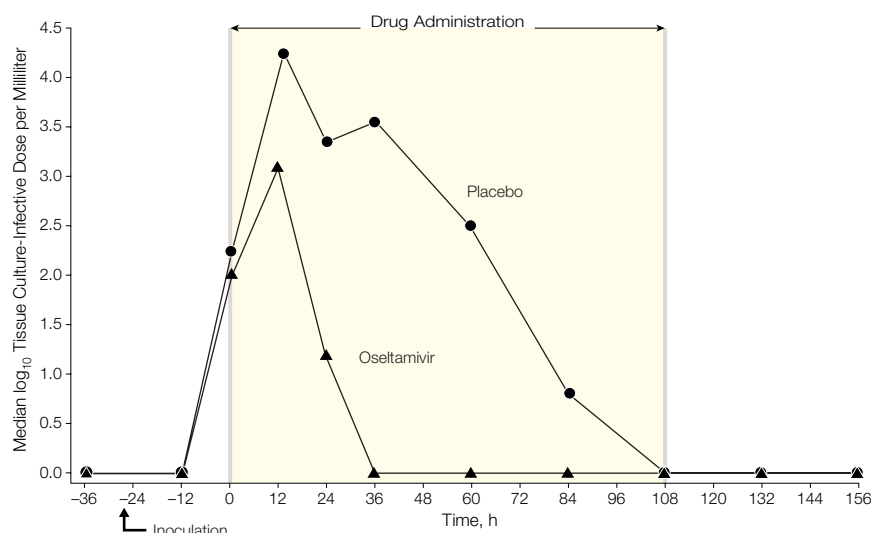
The viral titer area under the curve value was lower in the combined oseltamivir group (n = 56) compared with placebo (n = 13); $P = .02$.

Table. Dose-Related Antiviral and Clinical Effects of Early Oseltamivir Treatment in Experimental Influenza A/Texas/36/91(H1N1) Infection*

Clinical Effects	Oseltamivir Dose					Placebo	P Value
	20 mg Twice Daily	100 mg Twice Daily	200 mg Twice Daily	200 mg Once Daily	Combined Oseltamivir		
No. of infected subjects	15	14	14	13	56	13	NA
Vital titer†‡	51 (16-162)	54 (8-105)	85 (48-128)	143 (39-160)	80 (23-151)	273 (79-306)	.02
Time to cessation of shedding, h	58 (23-83)	47 (34-59)	58 (35-59)	59 (35-83)	58 (35-59)	107 (83-131)	.003
Total symptom score†	231 (72-332)	232 (99-354)	209 (106-354)	235 (63-318)	225 (97-349)	400 (189-645)	.05
Nasal mucus weight, g	9 (7-13)	9 (5-12)	8 (6-10)	9 (6-17)	9 (6-13)	20 (9-29)	.02
No. (%) with a fever	2 (13)	2 (14)	1 (7)	3 (23)	8 (14)	4 (31)	NA

*Values listed as median (25%-75% interquartile range) unless otherwise specified. The data for vital titer, total symptom score, and nasal mucus weight reflect events after initiation of drug treatment. NA indicates not applicable. P values refer to comparisons of combined oseltamivir group with placebo group.
†Based on area under the curve.
‡Log_{10} median tissue culture-infective dose multiplied by hour per milliliter.

Figure 4. Effect of Oral Oseltamivir Treatment on Illness Following Experimental Influenza A/Texas/36/91(H1N1) Infection
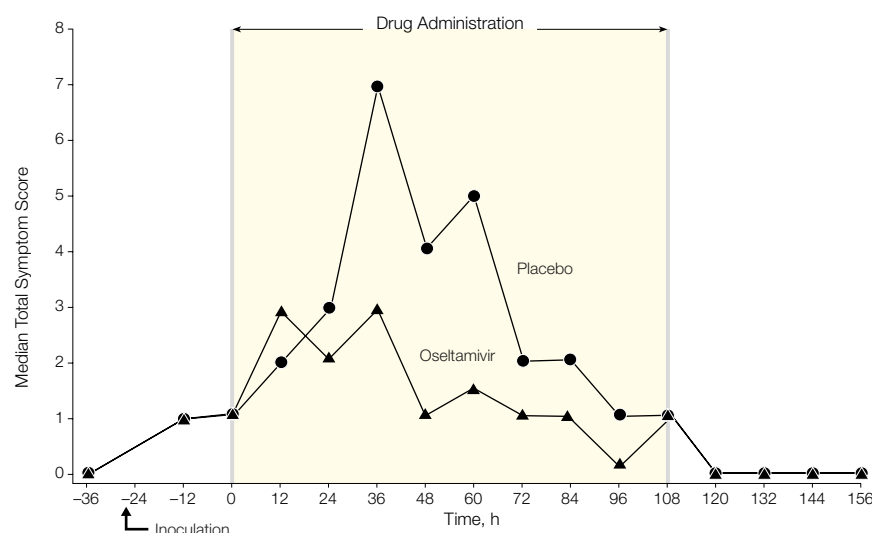
The total symptom score area under the curve value was lower in the combined oseltamivir groups (n = 56) compared with placebo (n = 13); P = .05.

piratory tract. Total symptom scores peaked at a lower level and resolved more rapidly in the combined oseltamivir group compared with placebo (**FIGURE 4**). The median time to resolution of illness was 53 hours in the oseltamivir group compared with 95 hours in the placebo group (P = .03). Nasal mucus weights were lower by approximately 50% in the oseltamivir group compared with placebo (P = .02) (Table). No important dose-related differences were noted in the principal clinical outcomes (Table). The frequencies of other measures including upper respiratory tract illness (18% vs 54%), middle ear pressure abnormalities (28% vs 54%), and fever (14% vs 31%) also were lower in oseltamivir recipients. Among oseltamivir recipients, fever occurred most often in the single daily dose group (23%) and least often in the 200-mg twice daily dose group (7%). Acetaminophen was used by 69% of placebo and 32% of oseltamivir recipients during the study for symptom relief, but the majority of subjects took only single doses. The frequency of cough was low in both groups (<10%).

Among infected placebo recipients, the median nasal lavage levels of IL-6, TNF-α, and IFN-γ exhibited similar patterns in that peak responses occurred on day 4 after inoculation in each instance (**FIGURE 5**). The magnitude of the median of the IL-6, TNF-α, and IFN-γ responses were comparable, exhibiting a 4-, 2- and 3-fold increase above baseline, respectively, on this day. By Spearman rank analysis, the increases in nasal lavage fluid of IL-6 and IFN-γ levels correlated significantly (P≤.01) with viral titers on days 3 (coefficients: IL-6, 0.71; IFN-γ, 0.78) and 5 (0.77 and 0.67, respectively) among placebo recipients. In contrast, oseltamivir treatment significantly reduced these responses, and no changes were observed in oseltamivir recipients over the course of the study. The IFN-α response did not show a significant elevation over the course of the infection, and there was no detectable IL-5 response in either group.

Tolerance

No dose-limiting intolerance was observed, and all subjects completed the dosing regimen. The overall frequency of adverse events during dosing did not differ across the groups, but gastrointestinal tract complaints occurred more often in the oseltamivir recipients (16 [18%] of 88 compared with 2 [7%] of 29 placebo recipients) during drug administration (95% confidence interval for difference, –11% to 68%). These were largely manifested as transient nausea of mild to moderate intensity (15 [17%] of 88 oseltamivir recipients). Emesis (4 [5%] of 88 oseltamivir recipients) and diarrhea (1 case)

were uncommon. Nausea occurred primarily after the first dose and usually resolved within 1 to 2 days despite continued dosing. In the treatment study, gastrointestinal tract upset was observed more often in subjects receiving 200-mg doses (9 [28%] of 32 compared with 3 [11%] of 32 receiving lower doses) and in the fasting state (11 [31%] of 36 compared with 2 [7%] of 28 subjects when administered with food). No significant changes were seen in clinical laboratory parameters (data not shown).

COMMENT

Oral administration of the neuraminidase inhibitor oseltamivir provided significant antiviral, biochemical, and clinical effects in experimental human influenza virus infection. Prophylactic administration either once or twice daily completely protected against viral recovery in the upper respiratory tract and against infection-associated respiratory tract illness. Early treatment resulted in statistically significant reductions in the magnitude and duration of viral replication and in the severity and duration of influenza illness. The magnitude of these effects is comparable with those observed earlier in studies of intranasal zanamivir tested against the same challenge virus under similar conditions.[2] In addition, the magnitude of the antiviral effects observed in these studies exceeded those reported historically with the influenza A–specific inhibitors amantadine and rimantadine in experimental human influenza.[26-31] Both topical zanamivir[10,11] and oral adamantaneamines[32-35] have been shown to provide clinical benefits in treating uncomplicated influenza, and prophylaxis with amantadine or rimantadine also provides substantial protection against epidemic influenza A illness.[36] Such findings predict that oral oseltamivir should be associated with both prophylactic and therapeutic activity when studied in natural influenza. Indeed, recently completed field studies have confirmed the efficacy of oral oseltamivir for both seasonal prophylaxis[37] and therapy of acute influenza.[38,39]

We recently reported that the nasal lavage fluid levels of IL-6 and TNF-α increase in humans experimentally infected with influenza A virus and correlate with the clinical and virological features of the illness.[24] In this study, we confirm these earlier observations and also document increases in levels of IFN-γ that parallel the increases in IL-6 and TNF-α. Among placebo recipients, the increases in levels of IL-6 and IFN-γ correlated with viral titers. This study verifies our previous findings of the role of IL-6, TNF-α, and IFN-γ in the pathogenesis of symptom production in influenza and shows that oral oseltamivir also reduced these objective markers of host inflammatory responses. These results further substantiate the links between virus replication, cytokine elaboration, and symptom production during acute influenza.

Oral oseltamivir was generally well-tolerated in these studies. Both GS4071 and zanamivir are selective inhibitors of influenza neuraminidase at low-nanomolar concentrations and show negligible activity against neuraminidases from human, bacterial, or other viral sources at concentrations up to 1 mmol/L.[14,15] In initial human testing, oral oseltamivir given while fasting was tolerated at single doses up to 1000 mg or repeated doses up to 500 mg twice daily for 7 days (total of 13 doses).[21] The highest doses, particularly repeated doses of 500 mg, were associated with nausea of mild to moderate intensity. In the current studies, transient nausea after dosing was observed mainly at the highest (200 mg) dose and was largely prevented by administering doses with food. The early timing of onset and the finding that ingestion with food largely prevented this adverse effect suggest that it might be due to direct gastric irritation, although further studies of the mechanism are needed. Of note, administration after food is associated with a modest increase in GS4071 bioavailability.[21] These observations suggest that this formulation of oseltamivir should be administered with food when possible to reduce the risk of gastrointestinal tract upset.

We did not observe clear dose-related differences in antiviral effects in the treatment or prophylaxis studies. Although once daily dosing may have been associated with less pronounced antiviral effects, our sample sizes were insufficient to make firm conclusions. With respect to prophylaxis, an antiviral dose that allows for a subclinical but immunizing infection would be optimal. Under similar conditions, intranasal zanamivir prophylaxis completely protected against experimental infection in most subjects, so that protective serologic responses were not observed.[2] With oseltamivir prophylaxis, both once and twice daily administration protected against viral shedding but allowed some

Figure 5. Median Nasal Lavage Fluid Levels of Interleukin 6, Interferon Gamma, and Tumor Necrosis Factor Alpha in Volunteers Experimentally Infected With Influenza A/Texas/36/91(H1N1)
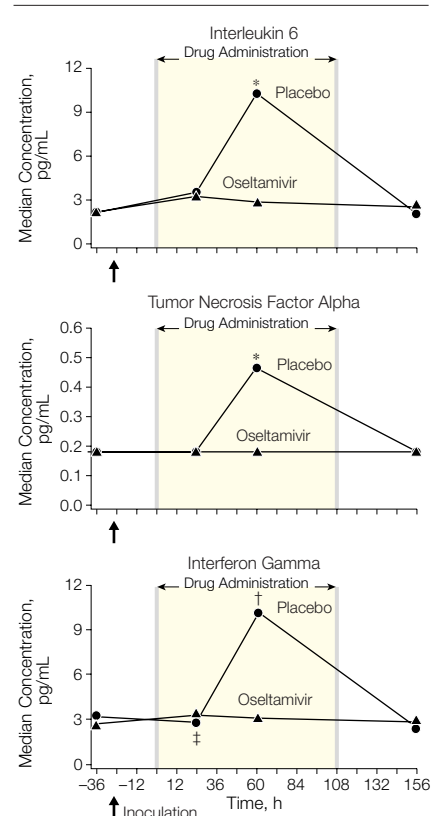
Cytokine levels for days 1, 3, 5, and 9 were determined using commercially available enzyme-linked immunosorbent assay kits. Asterisk indicates $P \leq .01$; dagger, $P \leq .001$; and double dagger, $P < .05$.

subjects to develop antibodies. Whether lower doses might be protective against illness but allow immune responses remains to be determined. Of note, the findings in these studies guided dose selection for subsequent field trials. The initial results from these studies indicate that once daily dosing (75 mg) is safe and effective for long-term prophylaxis [37] and twice daily dosing (75 mg) for short-term treatment of natural influenza in adults.[38,39]

In summary, the present studies showed oseltamivir to be an effective antiviral agent when administered orally for prevention and treatment of experimental influenza A infections. This agent is a promising new drug for the management of natural influenza.

Author Affiliations: University of Virginia School of Medicine, Charlottesville (Drs Hayden and Lobo); University of Rochester School of Medicine, Rochester, NY (Drs Treanor and Betts); SAIC Frederick, National Cancer Institute-Frederick Cancer Research and Development Center, Frederick, Md (Dr Fritz); Gilead Sciences, Foster City, Calif (Drs Miller and Mills); Roche Discovery Welwyn, Hoffman-LaRoche and Company, Welwyn Garden City, England (Mr Kinnersley and Dr Ward); and Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md (Dr Straus).

REFERENCES

1. Liu C, Eichelberger MC, Compans RW, Air GM. Influenza type A virus neuraminidase does not play a role in viral entry, replication, assembly, or budding. *J Virol.* 1995;69:1099-1106.
2. Hayden FG, Treanor JJ, Betts RF, Lobo M, Esinhart JD, Hussey EK. Safety and efficacy of the neuraminidase inhibitor GG167 in experimental human influenza. *JAMA.* 1996;275:295-299.
3. Burnet FM. Mucins and mucoids in relation to influenza virus action, III: inhibition of virus haemagglutination by glandular mucins. *Aust J Exp Biol Med Sci.* 1948;26:371-379.
4. Colman PM, Varghese JN, Laver WG. Structure of the catalytic and antigenic sites in influenza virus neuraminidase. *Nature.* 1983;303:41-44.
5. Colman PM. Influenza virus neuraminidase: structure, antibodies, and inhibitors. *Protein Sci.* 1994;3:1687-1696.
6. Varghese JN, McKimm-Breschkin JL, Caldwell JB, Kortt AA, Colman PM. The structure of the complex between influenza virus neuraminidase and sialic acid, the viral receptor. *Proteins.* 1992;14:327-332.
7. von Itzstein M, Wu WY, Kok GB, et al. Rational design of potent sialidase-based inhibitors of influenza virus replication. *Nature.* 1993;363:418-423.
8. Ryan DM, Ticehurst J, Dempsey MH, Penn CR. Inhibition of influenza virus replication in mice by GG167 (4-guanidino-2,4-dideoxy-2,3-dehydro-N-acetylneuraminic acid) is consistent with extracellular activity of viral neuraminidase (sialidase). *Antimicrob Agents Chemother.* 1994;38:2270-2275.
9. Ryan DM, Ticehurst J, Dempsey MH. GG167 (4-guanidino-2,4-dideoxy-2,3-dehydro-N-acetylneuraminic acid) is a potent inhibitor of influenza virus in ferrets. *Antimicrob Agents Chemother.* 1995;39:2583-2584.
10. Hayden FG, Osterhaus ADME, Treanor JJ, et al. Efficacy and safety of the neuraminidase inhibitor zanamivir in the treatment of influenza virus infections. *N Engl J Med.* 1997;337:874-879.
11. MIST Study Group. Randomized trial of efficacy and safety of inhaled zanamivir in treatment of influenza A and B virus infections. *Lancet.* 1998;352:1877-1881.
12. Cass LMR, Efthymiopoubs C, Bye A. Pharmacokinetics of zanomivir after intravenous, oral inhaled, or intranasal administration to healthy volunteers. *Clin Pharmacokinet.* 1999;36(suppl):1-11.
13. Cass LMR, Brown J, Pickford M, et al. Pharmacoscintigraphic evaluation of lung deposition of inhaled zanamivir in healthy volunteers. *Clin Pharmacokinet.* 1999;36(suppl):21-31.
14. Kim CU, Lew W, Williams MA, et al. Influenza neuraminidase inhibitors possessing a novel hydrophobic interaction in the enzyme active site: design, synthesis, and structural analysis of carbocyclic sialic acid analogues with potent anti-influenza

activity. *J Am Chem Soc.* 1997;119:681-690.
15. Mendel DB, Tai CY, Escarpe PA, et al. Oral administration of a prodrug of the influenza virus neuraminidase inhibitor GS4071 protects mice and ferrets against influenza infection. *Antimicrob Agents Chemother.* 1998;42:640-646.
16. Hayden FG, Rollins BS. In vitro activity of the neuraminidase inhibitor GS4071 against influenza viruses. *Antiviral Res.* 1997;34:A86.
17. Li W, Escarpe PA, Eisenberg EJ, et al. Identification of GS4104 as an orally bioavailable prodrug of the influenza virus neuraminidase inhibitor GS4071. *Antimicrob Agents Chemother.* 1998;42:647-653.
18. Sidwell RW, Huffman JH, Barnard DL, et al. Inhibition of influenza virus infections in mice by GS4104, an orally effective influenza virus neuraminidase inhibitor. *Antiviral Res.* 1998;37:107-120.
19. Kim CU, Bischofberger N, Williams MA, Lew W, Merson J, Sweet C. Efficacy of GS4104 in ferrets infected with influenza A virus. *Antiviral Res.* 1997;34:A74.
20. Eisenberg A, Bidgood A, Lynch G, Lee WA, Cundy K. Penetration of GS4071: a novel influenza neuraminidase inhibitor into rat bronchoalveolar lining fluid following oral administration of the prodrug GS4104. *Antimicrob Agents Chemother.* 1997;41:1949-1952.
21. Wood ND, Aitken M, Sharp S, Evison H. Tolerability and pharmacokinetics of the influenza neuraminidase inhibitor Ro-64-0802 (GS4071) following oral administration of the prodrug Ro-64-0796 (GS4104) to healthy male volunteers. In: Abstracts of the 37th Interscience Conference on Antimicrobial Agents and Chemotherapy; September 28-October 1, 1997; Toronto, Ontario. Abstract A-123.
22. Hayden FG, Tunkel AR, Treanor JJ, Betts RF, Allerheiligen S, Harris J. Oral LY217896 for prevention of experimental influenza A virus infection and illness in humans. *Antimicrob Agents Chemother.* 1994;38:1178-1181.
23. Walker JB, Hussey EK, Treanor JJ, Montalvo A, Hayden FG. Effects of the neuraminidase inhibitor zanamivir on otologic manifestations of experimental human influenza. *J Infect Dis.* 1997;176:1417-1422.
24. Hayden FG, Fritz RS, Lobo M, Alvord G, Strober W, Straus SE. Local and systemic cytokine responses during experimental human influenza A virus infection. *J Clin Invest.* 1998;101:643-649.
25. Santer TJ, Snell MK. Small-sample confidence intervals for P_1-P_2 and P_1/P_2 in 2×2 contingency tables. *J Am Stat Assoc.* 1980;75:386-394.
26. Sears SD, Clements ML. Protective efficacy of low-dose amantadine in adults challenged with wild-type influenza A virus. *Antimicrob Agents Chemother.* 1987;31:1470-1473.
27. Smorodintsev AA, Zlydnikov DM, Kiseleva AM, Romanov JA, Kazantsev AP, Rumovsky VI. Evaluation of amantadine in artificially induced A2 and B

influenza. *JAMA.* 1970;213:1448-1454.
28. Reuman PD, Bernstein DI, Keefer MC, Young EC, Sherwood JR, Schiff GM. Efficacy and safety of low dosage amantadine hydrochloride as prophylaxis for influenza A. *Antiviral Res.* 1989;11:27-40.
29. Jackson GG, Muldoon RL, Akers LW. Serological evidence for prevention of influenzal infection in volunteers by an anti-influenzal drug adamantanamine hydrochloride. *Antimicrob Agents Chemother.* 1963;3:703-707.
30. Dawkins ATJ, Gallager LR, Togo Y, Hornick RB, Harris BA. Studies on induced influenza in man, II: double-blind study designed to assess the prophylactic efficacy of an analogue of amantadine hydrochloride. *JAMA.* 1968;203:1095-1099.
31. Hayden FG, Zlydnikov DM, Iljenko VI, Padolka YV. Comparative therapeutic effect of aerosolized and oral rimantadine HCl in experimental human influenza A virus infection. *Antiviral Res.* 1982;2:147-153.
32. Hayden FG, Monto AS. Oral rimantadine hydrochloride therapy of influenza A virus H3N2 subtype infection in adults. *Antimicrob Agents Chemother.* 1986;29:339-341.
33. Hayden FG, Sperber SJ, Belshe RB, Clover RD, Hay AJ, Pyke S. Recovery of drug-resistant influenza A virus during therapeutic use of rimantadine. *Antimicrob Agents Chemother.* 1991;35:1741-1747.
34. Togo Y, Hornick RB, Felitti VJ, et al. Evaluation of therapeutic efficacy of amantadine in patients with naturally occurring A2 influenza. *JAMA.* 1970;211:1149-1156.
35. VanVoris LP, Betts RF, Hayden FG, Christmas WA, Douglas RGJ. Successful treatment of naturally occurring influenza A/USSR/77 H1N1. *JAMA.* 1981;245:1128-1131.
36. Douglas RGJ. Prophylaxis and treatment of influenza. *N Engl J Med.* 1990;322:443-450.
37. Hayden FG, Atmar RL, Schilling M, et al. Safety and efficacy of oral GS4104 in long-term prophylaxis of natural influenza. In: Final Program, Abstracts and Exhibits Addendum: 38th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy; September 24-27, 1998; San Diego, Calif. Abstract LB-6.
38. Treanor JJ, Vrooman PS, Hayden FG, Kinnersley N, Ward P, Mills RG. Efficacy of oral GS4104 in treating acute influenza. In: Final Program, Abstracts and Exhibits Addendum: 38th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy; September 24-27, 1998; San Diego, Calif. Abstract LB-4.
39. Aoki FY, Osterhaus AD, Rimmelzwaan GF, Kinnersley N, Ward P. Oral GS4104 successfully reduces duration and severity of naturally acquired influenza. In: Final Program, Abstracts and Exhibits Addendum: 38th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy; September 24-27, 1998; San Diego, Calif. Abstract LB-5.