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Safety and Efficacy of Intravenous Zanamivir in Preventing Experimental Human Influenza A Virus Infection

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Zanamivir is a potent inhibitor of influenza A and B virus neuraminidases and is active topically in experimental and natural human influenza. We conducted this double-blinded, placebo-controlled study to evaluate the safety and efficacy of intravenously administered zanamivir. Susceptible volunteers were randomized to receive either saline or zanamivir (600 mg) intravenously twice daily for 5 days beginning 4 h prior to intranasal inoculation with ~10^5 50% tissue culture infectious doses ($TCID_{50}$) of influenza A/Texas/36/91 (H1N1) virus. Reductions in the frequency of viral shedding (0% versus 100% in placebo, $P < 0.005$) and seroconversion (14% versus 100% in placebo, $P < 0.005$) and decreases in viral titer areas under the curve (0 versus 11.6 [median] $\log_{10}$ $TCID_{50}$ · day/ml in placebo, $P < 0.005$) were observed in the zanamivir group, as were reductions in fever (14% versus 88% in placebo, $P < 0.05$), upper respiratory tract illness (0% versus 100% in placebo, $P < 0.005$), total symptom scores (1 versus 44 [median] in placebo, $P < 0.005$), and nasal-discharge weight (3.9 g versus 17.5 g [median] in placebo, $P < 0.005$). Zanamivir was detectable in nasal lavage samples collected on days 2 and 4 (unadjusted median concentrations, 10.5 and 12.0 ng/ml of nasal wash, respectively). This study demonstrates that intravenously administered zanamivir is distributed to the respiratory mucosa and is protective against infection and illness following experimental human influenza A virus inoculation.

Influenza virus neuraminidase is a surface glycoprotein with enzymatic activity that catalyzes the cleavage of the linkage between a terminal sialic acid and an adjacent sugar residue. This action promotes the release of virions from infected cells, prevents viral aggregation after release from infected cells, prevents viral inactivation by respiratory mucus, and promotes viral spread through the respiratory tract mucosa (2). Zanamivir (4-guanidino-2,3-dideoxy-2,3-dehydro-*N*-acetylneuraminic acid; also called GG167) is a potent and selective inhibitor of the neuraminidases of influenza A and B viruses (4, 7, 12, 14). It is efficacious in shortening the duration and decreasing the severity of experimental infections in animals and humans when given by the intranasal route (8, 10, 11, 13). Inhaled zanamivir is therapeutically active in acute, uncomplicated, naturally occurring human influenza (1, 6, 9). Intranasal zanamivir is also efficacious in preventing experimental human influenza virus infection when given before virus inoculation (5, 8). Zanamivir inhaled once-daily (at a dose of 10 mg) has recently been shown to be an effective form of prophylaxis against naturally occurring influenza illness in young adults (9a).

Most efficacy studies to date have used topically administered zanamivir. This is because early pharmacokinetics studies showed poor oral bioavailability in human volunteers (median bioavailability, 2%; range, 1 to 5%) (3a). Although intravenous doses provided the highest peak concentrations in serum in human volunteers, the drug was rapidly eliminated in an unmetabolized form (3). Zanamivir has an extracellular site of action, and a topical application could provide a higher drug concentration at the site of infection. A high local concentration of zanamivir may potentiate its antiviral effects and possibly reduce the potential for the development of resistance. However, systemic administration of zanamivir warrants consideration as a form of administration which could be used in patients for whom inhalation may be difficult or may not effectively deliver the drug to sites of viral replication (as in cases of pneumonic disease).

Despite earlier animal studies showing no significant reductions in lung homogenate viral titers after the oral administration of zanamivir (10), other studies have found that antiviral effects occur following oral administration of the drug (11). When mice were given oral doses of at least 1 mg/kg/day of body weight 4 h prior to inoculation with influenza A/NWS/33 (H1N1) virus, increases in the mean number of days to death and in oxygen saturation were seen. In those mice given doses of 10 mg/kg, a significant reduction in mortality was observed.

Animal studies have recently shown that systemically administered zanamivir does have demonstrable antiviral effects. In a mouse model, intraperitoneal doses as low as 1 mg/kg/day had antiviral activity (3a). Multiple intravenous doses of zanamivir (600 mg twice daily for 5 days) were well tolerated in 12 uninfected human volunteers (3a). Consequently, this study was designed to determine the safety and tolerability of repeated intravenous doses of zanamivir and its efficacy in modifying viral replication and preventing illness in experimental human influenza virus infection.

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MATERIALS AND METHODS

Volunteers. The subjects were healthy, young adult males serologically susceptible to the challenge virus (serum hemagglutination inhibition [HI] antibody titers ≤1:8). Subjects were confined to the General Clinical Research Center at

the University of Virginia Health Sciences Center for 8 consecutive days, beginning 1 day prior to inoculation with the challenge virus. Each subject provided written informed consent on a form approved by the University of Virginia institutional review board. Subjects were compensated for their participation.

Experimental design. This was a randomized, placebo-controlled, double-blinded study designed to investigate the safety and efficacy of zanamivir, administered intravenously in repeated doses, in preventing illness and reducing viral replication in experimental human influenza virus infection. Subjects received either a placebo (saline) or zanamivir (600 mg) infused intravenously over 30 min twice daily (approximately every 12 h) for 5 days, beginning 4 h prior to inoculation with the challenge virus. This study was designed to test whether zanamivir given intravenously to human subjects provided antiviral effects in the respiratory tract. The administration of high doses of the drug (600 mg is the highest intravenous dose evaluated to date in human subjects) initiated prior to inoculation was done to increase the likelihood of detecting such an effect. Drug administration was begun 4 h prior to virus exposure to be consistent with the design of earlier studies of intranasal zanamivir (5, 8). All subjects were inoculated intranasally with $\sim 10^5$ 50% tissue culture infectious doses ($TCID_{50}$) of influenza A/Texas/36/91 (H1N1) virus provided by the National Institute of Allergy and Infectious Diseases, Bethesda, Md., by following a previously described protocol (8). The earlier study found that this virus inoculum results in infection rates of more than 70%. For this particular virus, the average 50% inhibitory concentration of zanamivir was 0.02 μg/ml as determined by plaque assay in Madin-Darby canine kidney (MDCK) cells (7).

Each subject completed a symptom assessment score sheet twice daily throughout the confinement period. As previously described (8), this scoring system assessed 14 symptoms that were rated as absent (0 points), mild (1 point), moderate (2 points), or severe (3 points), with a maximum possible score of 42 points per assessment period. Vital signs, including temperature, were obtained twice daily. Daily nasal-discharge weights were determined by the collection of preweighed tissues in preweighed plastic bags assigned to each subject during each 24-hour period. Nasal washes were obtained daily for viral recovery in MDCK cell monolayers by standard techniques, and titers of the infectious virus were determined by culturing serial 10-fold dilutions of once-frozen (−70°C) samples that were positive for virus on initial isolation. Zanamivir concentrations in nasal washes were determined by liquid chromatography-mass spectrometry with a lower quantifiable limit of 5 ng/ml on days 2 and 4 prior to the morning dose. Monitoring of subjects for adverse events was performed throughout the confinement period by asking the subjects to report any symptoms that they experienced. Laboratory parameters, including blood chemistries, a complete blood count with differential, and a urinalysis, were determined for each subject at screening (within 21 days of inoculation), prior to the first dose of the study drug, and on day 7.

Data analysis. Infection was defined as the isolation of the challenge virus from one or more nasal wash samples and/or a ≥4-fold rise in serum HI antibody titers at 3 to 4 weeks after inoculation. Fever was defined as an oral temperature greater than 37.7°C. Upper respiratory tract illness (URI) was defined as two or more upper respiratory tract symptoms (nasal stuffiness, runny nose, sore throat, sneezing, hoarseness, and ear pressure or earache) of any severity for 2 or more days. Comparisons of the proportions of events (viral shedding, seroconversion, infection, and clinical outcome) between the placebo and zanamivir groups were made by using Fisher's exact test. Comparisons of symptom scores and nasal-discharge weights were performed by using the rank sum test. All analyses were performed with SAS software (version 6.12). Protective efficacy was defined as follows: [(rate in placebo group − rate in zanamivir group)/rate in placebo group] × 100. Based on prior experience with this challenge virus (8), the sample size was set as eight subjects per group to detect a 1.5-$\log_{10}$ difference in the area under the curve (AUC) for viral titers, with 80% power ($\alpha = 0.05$).

RESULTS

Volunteers. Sixteen male volunteers were enrolled in the study. Demographic characteristics in the two groups were comparable (Table 1). All 16 subjects completed the study and were included in the safety analyses. One subject in the zanamivir group was excluded from the efficacy analyses due to the isolation of a nonchallenge virus (rhinovirus) from a preinoculation sample.

Drug levels in nasal washes. The median (range) nasal-wash zanamivir concentrations, not adjusted for the dilution factor resulting from the sampling technique, were 10.5 ng/ml (<5 to 36 ng/ml) and 12 ng/ml (<5 to 20 ng/ml) on days 2 and 4, respectively.

Antiviral activity. All eight subjects receiving the placebo treatment shed virus after inoculation and had ≥4-fold increases in HI antibody titers at the 3- to 4-week follow-up. In the group receiving zanamivir ($n = 7$), no viral shedding was detected ($P < 0.005$). Serologic evidence of infection with the challenge strain was detected in only 14% (1 of 7) of subjects in the zanamivir group ($P < 0.005$). Thus, the protective efficacies of intravenous zanamivir against viral shedding and seroconversion were 100 and 86%, respectively. Figure 1 shows the nasal-wash viral titers for the two groups on each day of the study. The placebo group showed the expected peak in titers on the second day after challenge, at which time there was a 3.4-$\log_{10}$ difference in titers compared to the zanamivir group. The median AUC for nasal-wash viral titers (in $\log_{10}$ $TCID_{50}$ · day/ml) was 11.6 for the placebo group, compared to 0 for the zanamivir-treated subjects ($P < 0.005$).

TABLE 1. Demographic characteristics of treatment groups[a]

Characteristic	Value for group	
	Zanamivir	Placebo
Female/male	0/8	0/8
Race, no. (%)		
Black	1 (12.5)	1 (12.5)
Caucasian	7 (87.5)	7 (87.5)
Age, yr		
Median (range)	22 (18–33)	20.5 (19–35)
Mean (SD)	23.9 (5.4)	24.1 (7.0)
Wt, kg		
Median (range)	68.7 (63–94.8)	79.3 (61.7–112)
Mean (SD)	71.3 (10.1)	80.9 (15.2)
Ht, cm		
Median (range)	174.5 (170–191)	180 (170–189)
Mean (SD)	177.8 (7.3)	180 (7.4)
Body mass index, kg/m^2		
Median (range)	22.1 (18.8–27.4)	24.8 (20.1–31.4)
Mean (SD)	22.6 (2.74)	24.9 (3.46)

[a] These demographic characteristics describe the eight subjects initially randomized to each group. There were no significant differences between the groups.

Illness measures. Zanamivir administration produced significant reductions in fever and multiple other measures of illness severity (Table 2). Seven (87.5%) of the eight placebo subjects had fever, while only one (14.3%) of the seven subjects in the zanamivir-treated group developed fever (protective efficacy, 84%; $P < 0.05$). Of note, the one fever that occurred in the zanamivir group was low grade (37.9°C), was present at only one time point, was not associated with respiratory illness, and did not occur in the one subject who was later found to have serologic evidence of infection with the challenge strain. All placebo-treated subjects developed URI, compared to none of the zanamivir-treated volunteers (protective efficacy, 100%; $P < 0.005$). Myalgia did not occur in the zanamivir-treated group, while five (63%) of the placebo recipients did experience myalgia ($P < 0.05$). Figure 2 shows the median total symptom score for each study group, determined by symptom assessment on a twice-daily basis. The typical peak in symptoms on days 2 and 3 after challenge that was seen in the placebo group was markedly attenuated by intravenous zanamivir. The zanamivir group also had significantly lower median total symptom scores than the placebo group (Table 2). Similarly, the median nasal-discharge weights were 78% lower in zanamivir recipients.

Safety. All 16 subjects were included in the safety analyses. No subjects withdrew from the study, and no serious adverse events occurred. Three adverse events classified as severe were documented. Two of these events occurred in the placebo group. One subject reported severe overall discomfort beginning 2 days after the initial dosing. This resolved within 3 days

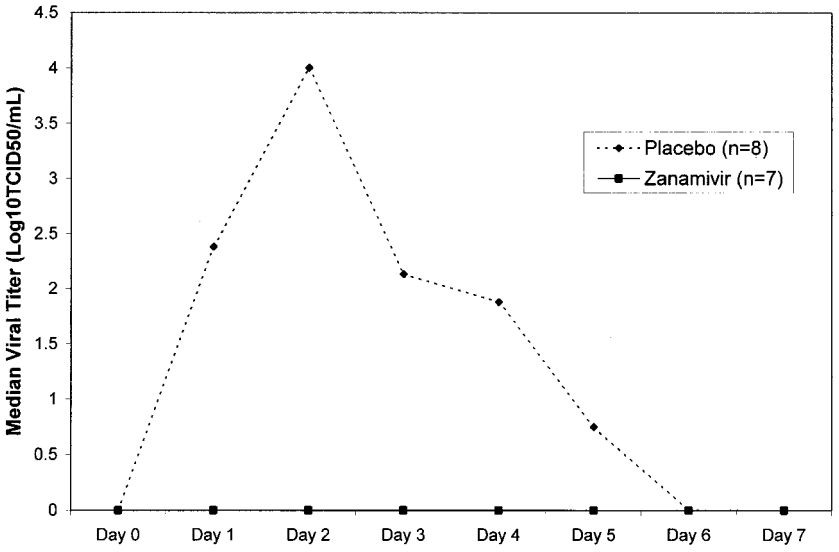

FIG. 1. Titers of influenza A virus in nasal washes collected from volunteers experimentally infected with influenza A/Texas/36/91 virus following administration of intravenous zanamivir or placebo twice daily. Drug administration began 4 h prior to inoculation on day 0 and continued for 5 days. There was a significant difference in the AUC values for viral titers between the placebo (11.6 $\log_{10}$ $TCID_{50}$ · day/ml) and zanamivir (0) ($P < 0.005$) groups.

of its onset. The other event was a severe headache which resolved within 3 h. The one severe event reported in the zanamivir group was a URI that occurred 15 days after the last dosing. It was felt that these occurrences were not related to the study drug. The other reported complaints were consistent with influenza-produced illness. No alterations in the hematology or chemistry profiles of zanamivir recipients occurred. The only abnormality detected in the placebo group was an elevated γ-glutamyltransferase level in one subject on day 7.

DISCUSSION

This study provides clear evidence that intravenously administered zanamivir is well tolerated and protective against infection and illness due to experimental human influenza A virus exposure. The prophylactic administration of intravenous zanamivir completely protected against viral recovery and markedly reduced the rate of seroconversion in treated subjects compared to placebo recipients. Significant reductions were seen in all clinical markers of illness evaluated, except cough. Reductions in cough were not expected because of the low incidence of lower respiratory tract disease in experimental influenza virus infection in humans. This is indicated by the relatively low number of subjects in the placebo group (25%) that developed cough, despite a high rate of occurrence of the other clinical markers in this group. The findings of this study indicate that zanamivir is distributed to respiratory tract secretions in an antivirally active form after intravenous administration in humans. The finding that intravenous zanamivir was highly protective against viral infection is consistent with earlier studies of intranasal zanamivir (8). Of note, the levels of protection against viral shedding and infection (100 and 86%, respectively) observed in this small study of intravenous zanamivir are similar to those observed when multiple intranasal doses of zanamivir were given for prophylaxis of experimental A/Texas/36/91 (H1N1) infection (96 and 82%, respectively) (8). These results suggest an essential role for influenza virus neuraminidase in initiating viral infection within the respiratory tract, possibly by preventing the inactivation of virus by respiratory tract secretions and enabling initial infection to take place.

TABLE 2. Clinical outcome in evaluable subjects[a]

Characteristic or outcome	Value for group	
	Zanamivir	Placebo
No. of subjects	7	8
No. (%) with fever	1 (14)[b]	7 (88)
No. (%) with URI	0 (0)[c]	8 (100)
No. (%) with cough	0 (0)	2 (25)
No. (%) with myalgia	0 (0)[b]	5 (63)
Total symptom score		
Median (range)	1 (0–6)[b]	44 (20–101)
Mean (SD)	2 (2)[b]	51 (28.6)
Total nasal-discharge wt (g)		
Median (range)	3.9 (3.75–8.45)[c]	17.5 (4.35–55.6)
Mean (SD)	5.0 (1.8)[c]	19.2 (15.6)

[a] Fever was defined as an oral temperature of >37.7°C; URI was defined as ≥2 respiratory symptoms (nasal stuffiness, runny nose, sore throat, sneezing, hoarseness, and ear pressure or earache) of any severity for ≥2 days; for cough and myalgia, occurrence of any severity for ≥2 days was included in assessment of outcome.

[b] $P < 0.05$ compared to value for placebo.

[c] $P < 0.005$ compared to value for placebo.

The detection of zanamivir in nasal washes prior to morning dosing (10.5 ng/ml on day 2 and 12 ng/ml on day 4) supports this conclusion. These concentrations were not adjusted for the dilution factor, due to the nasal lavage procedure. In general, uncorrected values are approximately 10- to 20-fold lower than those adjusted for the dilution factor. Therefore, estimated adjusted zanamivir concentrations in nasal washes would be approximately 100 to 200 ng/ml 12 h postdose. These concentrations were similar to those found in a study of noninfected volunteers receiving the same dosing regimen, in whom the median dilution-corrected zanamivir concentrations were 99 and 133 ng/ml 12 h after the morning dose on days 1 and 5, respectively (3a). Given that the estimated 50% inhibitory concentrations for zanamivir are 0.64 to 7.9 ng/ml as determined by enzyme inhibition assays (14) and that zanamivir concen-

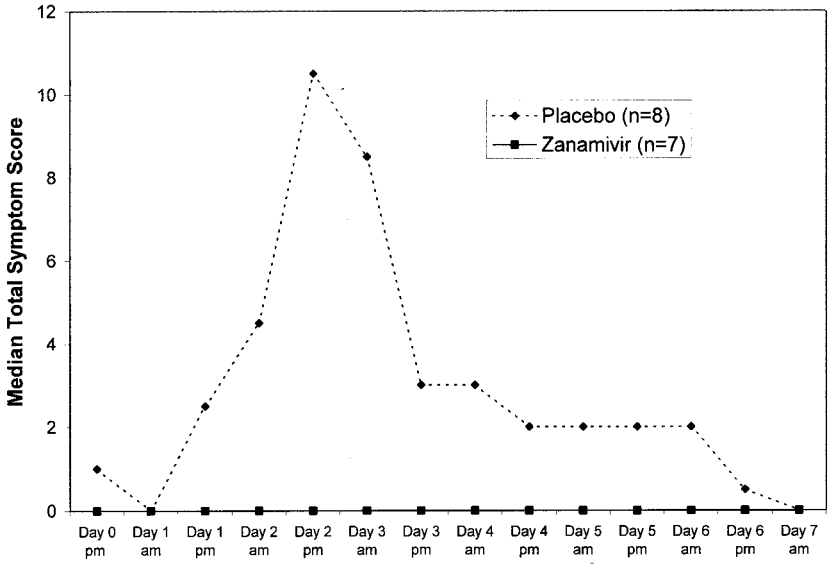

FIG. 2. Daily symptom scores of volunteers experimentally infected with influenza A/Texas/36/91 virus following administration of intravenous zanamivir or placebo twice daily. Drug administration began 4 h prior to inoculation on day 0 and continued for 5 days. There was a significant difference in median total symptom scores between the zanamivir (1 point) and placebo (44 points) groups ($P < 0.05$).

trations of 10 ng/ml have been shown to produce at least a 1.0-$\log_{10}$ $TCID_{50}$/ml decrease in yield for clinical influenza A virus isolates in human respiratory tract epithelial cells (7), it appears that zanamivir administered at the study dose would provide concentrations exceeding the enzyme inhibitory concentration throughout the treatment period.

One limitation of this study is that the first dose of zanamivir was administered prior to exposure to influenza virus, a situation unlikely to occur in clinical practice. However, this study was the first to evaluate the efficacy of intravenously administered zanamivir in humans and was thus designed to maximize the ability to detect antiviral activity. The findings of significant antiviral activity and tolerability in this optimal setting provide an impetus for the study of systemically administered zanamivir in established human influenza virus infection. In order to meet the medical need for effective antiviral treatment of severe influenza in hospitalized patients, the investigation of various formulations and combinations of compounds with anti-influenza activity is warranted. The use of nebulized zanamivir in patients hospitalized with lower respiratory tract infections due to influenza virus is currently being evaluated in a randomized controlled trial by the National Institutes of Health Collaborative Study Group. Further investigation of intravenously administered zanamivir, in combination with other formulations of the drug or other antiviral agents, in patients hospitalized with severe illness due to influenza virus may also be of value.

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