Efficacy and tolerability of the oral neuraminidase inhibitor peramivir in experimental human influenza: randomized, controlled trials for prophylaxis and treatment

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Objective: Oseltamivir is the only oral neuraminidase inhibitor currently available; we determined the tolerability and antiviral efficacy of oral peramivir for treatment and prophylaxis of experimental human influenza A and B.

Participants: 288 susceptible, healthy volunteers (ages 18–45) were inoculated intranasally with A/Texas/36/91/H1N1 or B/Yamagata/16/88 virus in four randomized, double-blind, placebo-controlled trials.

Interventions: For treatment dosing was initiated at 24 h after inoculation with peramivir doses ranging from 100–800 mg/day for 5 days. For prophylaxis dosing was initiated 24 h before inoculation and continued for 4 days with peramivir doses ranging from 50–800 mg/day.

Outcomes: The primary outcome measure for treatment was quantitative viral detection defined by the area under the curve (AUC) for nasal wash viral titres. For prophylaxis the primary outcome measure was the incidence of virus recovery.

Results: In influenza A treatment, peramivir 400 mg q24h and 200mg q12h, but not lower doses, resulted in significant reductions in viral titre AUC. In influenza B treatment, both 400 and 800/400 mg once daily dose groups reduced AUC values. In influenza A prophylaxis, the percentage of individuals with nasal viral shedding did not differ significantly in the placebo (58%), 50 mg (61%), 200 mg (37%) and 400 mg (31%) dose groups. In influenza B prophylaxis, shedding frequencies were similar in placebo (55%), 200 mg (41%), 400 mg (35%) and 800 mg (47%) dose groups. The drug was well tolerated in all four studies, with nausea and headache being the most common side effects. No drug-resistant variants were detected.

Conclusion: Early treatment with peramivir was associated with significant antiviral effects in experimentally induced influenza in humans. Prophylaxis did not significantly reduce viral shedding. The relatively low blood peramivir concentrations observed may explain the lack of more robust antiviral effects, and parenteral dosing should be studied.

Introduction

Influenza continues to cause considerable morbidity and mortality, particularly among the elderly and immunosuppressed individuals [1], and presents the ongoing threat of a new pandemic [2]. While immunization remains the primary means of prevention, antiviral drugs are an important adjunct, particularly for situations in which vaccine is unavailable or ineffective due to viral antigenic changes or poor host immune response. Although useful agents, the M2 channel inhibitors amantadine and rimantadine are limited by central nervous system (CNS) and gastrointestinal side effects, the emergence of viral resistance, and the inherent resistance of influenza B due to its lack of an M2 protein [3]. Of note, many recent human isolates of avian H5N1 virus have shown resistance to this class of antiviral drugs [4].

The neuraminidase inhibitors (NAIs) are an important advance in the management of influenza with respect to activity against influenza A and B viruses, proven therapeutic value in reducing influenza lower respiratory complications [5], lack of CNS side effects, and lesser problems with antiviral drug resistance [6].

Both inhaled zanamivir and oral oseltamivir are effective in both prophylaxis and treatment of influenza A and B infections, although zanamivir is not currently approved for prophylaxis in the United States [6]. The use of these agents has been limited by concerns regarding cost, degree of effectiveness, need for an inhaler device and risk of bronchospasm with zanamivir, and, in the case of oseltamivir, gastrointestinal side effects and emergence of resistant variants in some treated populations [6–8]. Oseltamivir is currently being stockpiled by many countries for use in the event of a major influenza outbreak or pandemic. The availability of another oral anti-influenza agent that could provide an alternative to oseltamivir would be important.

Peramivir (formerly RWJ-270201 or BCX 1812) is a novel cyclopentane NAI that, like oseltamivir, is orally bioavailable. The drug inhibits influenza A and B viruses *in vitro* [9,10] and in murine and ferret models of influenza [11–13]. The drug also shows a distinct *in vitro* resistance profile from zanamivir and oseltamivir, in that some viral variants with *in vitro* zanamivir or oseltamivir resistance retain at least partial susceptibility to peramivir [9,10]. The oral bioavailability of peramivir is lower than with oseltamivir, but the plasma elimination half-life, which averages approximately 14 h and ranges from about 9–30 h, is approximately twice as long (Investigator's Brochure RWJ-27021-162, October 2000, RW Johnson Research Institute). Consequently, the objectives of the current proof-of-concept studies were twofold: first, to ascertain the antiviral effectiveness of peramivir in early treatment and prophylaxis of experimental influenza infection in healthy volunteers; and, second, to assess its tolerability and safety. In addition, the relationships between dose and achieveable plasma concentrations over time were examined.

Methods

Participants

Participants in all studies were healthy adult volunteers aged 18–45 years, with screening serum hemagglutination-inhibition (HAI) antibody titre ≤1:8 to the challenge virus and willingness to use adequate birth control measures. Exclusion criteria varied slightly among studies but included respiratory illness within 1 week of study drug administration; use of steroid, immunosuppressant, or aspirin therapy; fever within 72 h of viral inoculation; previously experienced serious complications related to influenza; antiviral therapy within 2 weeks prior to study drug administration; a known allergy to eggs or components of viral inoculum; active asthma requiring chronic treatment; treatment with drugs known to effect gastrointestinal motility and renal excretion within 2 weeks prior to study drug; pregnancy or breast-feeding. All volunteers underwent a screening history and physical with baseline laboratory tests and ECG tracings. They were isolated in individual hotel rooms from the time of inoculation until 8 or 9 (influenza B) days afterwards. Participants signed an informed consent form approved by the respective institutions institutional review committees and were compensated for participation.

Design

The studies were randomized, double-blind, and placebo controlled in design and involved two centres (the University of Virginia and the University of Rochester). A computer-generated, block randomization schedule was used to assign treatment. Safety-tested pools of influenza A/Texas/36/91(H1N1) and influenza B/Yamagata/16/88 (provided through the National Institute of Allergy and Infectious Diseases) were used for intranasal inoculation (0.25 ml per nostril) by previously described methods [14–16]. The total inoculum of the challenge strain was targeted to be 10^6 50% tissue-culture infectious doses ($TCID_{50}$) per individual for influenza A and 10^7 $TCID_{50}$ for influenza B.

Treatment

One study with influenza A/Texas/36/91 and a second with B/Yamagta/16/88 were performed during 1999. On day 0 individuals were inoculated with virus, and treatment was begun 24 h later and given for 5 days. In the influenza A study, participants were randomly assigned to one of five treatment regimens: placebo and peramivir 100 mg q24h, 200 mg q24h, 400 mg q24h, or 200 mg q12h. The administered viral inoculum, based on standard back-titration of the diluted inoculum at the time of challenge, was determined to be $10^{6.2}$ and 10^7 $TCID_{50}$ per volunteer at the two respective centres. In the influenza B study, participants were randomly assigned to one of three treatment regimens: placebo, peramivir 800 mg (day 1) then 400 mg q24h or peramivir 400 mg q24h. Back-titrations indicated that the inocula administered were $10^{6.9}$ and $10^{7.5}$ $TCID_{50}$ per individual at the two respective centres.

Prophylaxis

Two studies, first with influenza A/Texas/36/91 and second with B/Yamagata/16/88 virus, were conducted in 1999 and 2000. Administration of the study drug was initiated 1 day before viral inoculation and continued for a total of 5 days. In the influenza A study, the groups included placebo and peramivir 50 mg q24h, 200 mg q24h, or 400 mg q24h. In the influenza B study, the treatment groups were placebo

and peramivir 200 mg q24h, 400 mg q24h, or 800 mg q24h. Individuals were inoculated intranasally with influenza A/Texas/36/91 (total inoculum $10^{5.9}$ per individual) or influenza B/Yamagta/16/88 (total inoculum $10^{6.3}$ to $10^{6.6}$ $TCID_{50}$ per individual).

Virological and clinical monitoring

A nasal wash was performed before viral inoculation (day -1) in the treatment studies to detect pre-existing viral infections; after inoculation nasal washings were performed twice daily on days 1–3 and once daily on days 4–7 for influenza viral culture and titration by previously described techniques [14,16]. In the prophylaxis studies, nasal washings were collected on day 1 before drug initiation and then every 24 h on days 2 through 8 or 9. All viral isolates recovered from individuals who shed virus for at least 3 days had the last isolate tested for susceptibility to peramivir by a neuraminidase inhibition assay [17,18].

Symptoms were recorded twice daily during the isolation period. Nasal mucus weights were determined during the isolation period by collecting pre-weighed tissues used for nose blowing into plastic bags. Safety evaluation, including vital signs, haematology, chemistry and urinalysis, were performed at baseline, during treatment and at discharge. In addition, in the prophylaxis studies, blood samples for pharmacokinetics were collected prior to drug administration on day 1, days 3–5, then 1, 3, 12 and 24 h after the final dose on day 5. Individuals returned to the study site between days 21 and 28 for collection of convalescent sera and follow-up.

Outcomes

Infection was defined as a positive viral culture for the challenge strain on one or more days after inoculation, by a fourfold or greater rise in serum HAI antibody titres to the challenge strain, or both. The primary outcome in the treatment studies was the reduction in quantitative viral shedding determined by cultures of nasal washes from infected individuals. Quantitative viral shedding was defined as the area under the viral titre/time curve from day 1 up to and including day 7 ($AUC_{days1–7}$). Included in the calculation are data from nine individuals in the active treatment group who did not shed virus but later were found to have seroconverted to the challenge virus. For purposes of analysis, the duration of viral shedding in these individuals was 0 days.

The primary efficacy evaluation in the prophylaxis studies was the incidence of viral shedding, defined as the proportion of individuals with at least one positive culture for the challenge strain in nasal washings collected from day 3 to day 8.

In both studies, secondary efficacy evaluations included duration and quantity of viral shedding, incidence of infection, incidences of fever and of upper respiratory tract illness and cough, symptom scores, and nasal mucus discharge weights. Fever was defined as oral temperature ≥38.0 °C. The composite symptom score consisted of the cumulative total score for seven symptoms (feverishness, headache, muscle aches, sore throat, cough, overall discomfort and nasal stuffiness/runny nose) rated on a four-point scale (absent, mild, moderate or severe), for the week after inoculation. Cough was defined as symptoms of cough of any severity that was present for at least 2 days after challenge. Upper respiratory illness (URI) was defined by at least two respiratory symptoms (cough not included) present for at least 2 days between days 2 and 8.

Statistical analyses

In the treatment studies, the primary efficacy variable was determined using an analysis of covariance (ANCOVA) model that included terms for investigative site and treatment as predictors and baseline titre as a covariate. Treatment-by-baseline titre interaction was assessed for inclusion/exclusion in the final ANCOVA model using a significance level of 0.1. The Wilcoxon exact test was used to compare the duration of viral shedding between groups. The AUC of composite and total symptom scores and the AUC nasal mucus discharge weight was analysed using an ANCOVA model, including terms for investigative site, treatment, baseline and treatment-by-baseline interaction. The Fisher exact test was used to compare the proportion of individuals with fever, and the Log-rank test for survival (Kaplan–Meier) analysis (stratified by investigative site with treatment as the predictor variable) was used to compare the time to alleviation of composite symptoms.

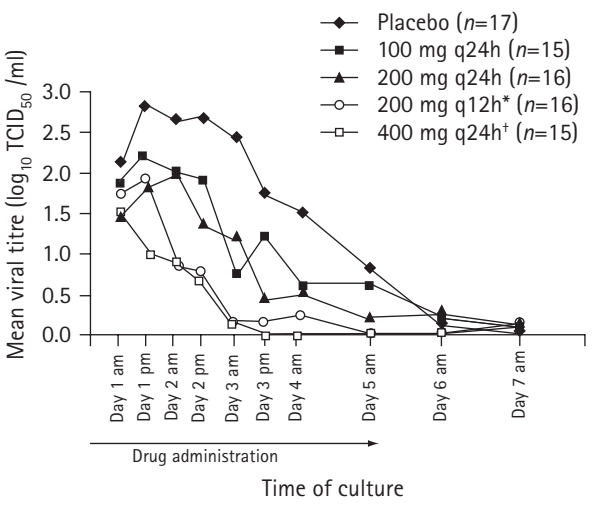
In the prophylaxis studies, the one-sided Fisher exact test was used to compare the incidence of viral shedding between active and placebo groups. A step-down testing procedure was used to control for the Type 1 error rate in multiple comparisons between treatments and to determine the lowest dose of active drug that was significant when compared with placebo.

The Fisher exact test was also used to compare the incidences of infection, URI and cough between the groups. The Wilcoxon–Gehan test for censored data was used to compare the durations of viral shedding. The differences between the active and placebo groups in peak $AUC_{days\ 2–8}$ ($AUC_{days\ 2–9}$ for influenza B) of viral titre and the peak $AUC_{days\ 3–8}$ ($AUC_{days\ 3–9}$ for influenza B) of nasal mucus discharge weight was tested for using an ANOVA, using only treatment as a term in the model.

Sample size estimates

In previous prophylaxis studies with two NAIs [15,16], the combined proportion of individuals shedding virus in the active and placebo groups was 2% and 64%, respectively. The proportion of individuals shedding virus in the current studies was assumed to be 60% in the placebo groups, and 10% in the treatment groups. The prophylaxis studies using a one-sided Fisher exact test with a significance value of 0.025 yielded a power of approximately 90% with twenty individuals per treatment group. The treatment studies used a two-sided Fisher exact test with a significance level of 0.05; the sample size was based on the assumption that the standard deviation of the viral titre AUC would range between 4 and 6 $\log_{10}$ $TCID_{50}$ × day/ml. It was further assumed that 80% of individuals in the influenza A study and 60–70% of individuals in the influenza B study would have confirmed infection, yielding a final sample size of twenty individuals per treatment group.

Figure 1. Mean nasal wash viral titres over time in treatment of experimental influenza A/Texas/36/91(H1N1) infection



*P<0.05; †P<0.001, peramivir versus placebo for comparison of viral titre area under the curve values. See Table 1. $TCID_{50}$, 50% tissue-culture infectious doses.

Results

Treatment – influenza A study

Ninety volunteers were randomized to treatment, with one participant withdrawing before receiving a dose of study medication, so that 89 individuals completed the study and were evaluable for safety. Seventy-nine (89%) individuals demonstrated laboratory confirmed infection and were evaluated for efficacy. The overall group consisted of 52% men/48% women. The age range was 18–45 years, with a median age of 22 years.

Both 400 mg once daily and 200 mg q12h peramivir dosing groups showed significant reductions in mean viral titre AUC values compared with placebo, whereas the lower dose groups showed lesser, non-significant antiviral effects (Figure 1 and Table 1). Compared with the other groups, the 400 mg per day regimen was associated with the most prompt fall in viral titres, a lower peak viral titre (approximate 2 $\log_{10}$ reduction compared with placebo), and shorter duration of viral shedding (approximately 2-day reduction compared with placebo; Table 1). Lesser but significant effects were noted in the 200 mg q12h group.

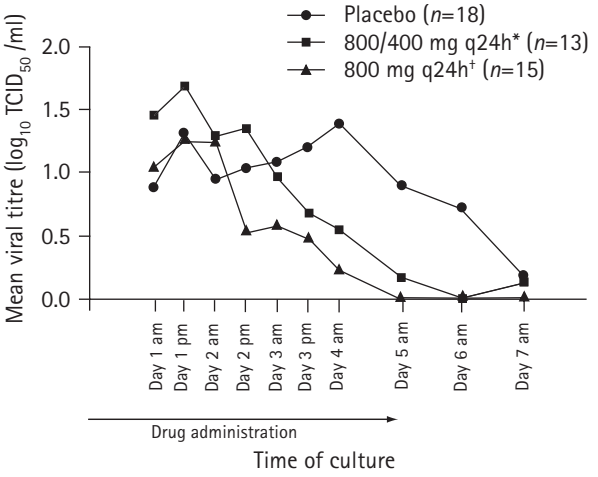
However, no significant differences were noted between the active treatment and placebo groups in symptom scores. In part this was due to the low

Table 1. Antiviral and clinical effects of early peramivir treatment in experimental A/Texas/36/91(H1N1) influenza virus infection

Outcomes	100 mg qd (*n*=18)	200 mg qd (*n*=18)	400 mg qd (*n*=18)	200 mg q12h (*n*=17)	Placebo (*n*=18)
Infected individuals, *n* (%)	15 (83)	16 (89)	16 (89)	15 (88)	17 (94)
Shedding virus, *n* (%)	13 (72)	13 (72)	13 (72)	14 (82)	17 (94)
Viral titre AUC*† mean (SE)	5.4 (1.1)	5.4 (1.0)	2.2 (1.0; *P*< 0.001)	2.6 (1.1; *P*=0.014)	8.2 (1.0)
Peak viral titre, $TCID_{50}$/ml (SE)	2.7 (0.4)	2.5 (0.4; *P*=0.047)	1.5 (0.4; *P*< 0.001)	2.2 (0.4; *P*=0.011)	3.6 (0.4)
Duration of viral shedding, mean days (SD)	2.7 (1.9)	2.6 (2.2)	1.0 (0.8; *P*=0.002)	1.8 (1.4)	3.2 (2.0)
Cumulative nasal mucus weight, mean g (SE)*	9.2 (4.8; *P*=0.041)	16.0 (4.3)	8.8 (4.3, *P*=0.025)	15.4 (4.6)	22.4 (4.3)
Individuals with fever, *n* (%)	1 (7%)	1 (6%)	0	0	4 (24%)
Composite symptom score‡, mean (SE)	5.6 (1.9)	6.4 (1.8)	6.1 (1.8)	6.1 (1.9)	8.9 (1.8)

*Based on area under the curve $(AUC)_{days\ 1–7}$. †Log_{10} 50% tissue culture-infectious doses per ml × day. ‡Based on $AUC_{days\ 1–7}$. SD, standard deviation; SE, standard error; qd, once daily.

Figure 2. Mean nasal wash viral titre over time in early treatment of experimental influenza B/Yamagata/16/88 infection



*P=0.026. †P=0.004, peramivir versus placebo for comparison of viral titre area under the curve values. See Table 2. $TCID_{50}$, 50% tissue-culture infectious doses.

frequency of symptoms in the placebo group, as the mean composite scores were low for all treatment groups. A retrospective analysis of pooled data from all treatment groups showed a significant difference in the incidence of fever between the combined peramivir treatment groups and placebo group (24% vs 3%, P=0.019). Nasal mucus weights were reduced by over one-half in the 100 mg once daily and 400 mg once daily groups (Table 1).

Treatment – influenza B study

Among 56 volunteers randomized, one participant withdrew before viral inoculation and receiving study medication, so that 55 individuals completed the study; 46 (84%) demonstrated laboratory confirmed infection and were evaluated for efficacy. The overall group consisted of 63% men and 37% women. The age range was 18–45 years, with a median age of 25 years.

Peramivir treatment was associated with significant reductions in mean viral titre over time in both groups compared with placebo (Figure 2 and Table 2). Similar to the influenza A study, trends favouring peramivir were seen for secondary virological endpoints, but none reached statistical significance (Table 2). The frequency and severity of illness were too low to discern differences between the groups.

Prophylaxis – influenza A study

Seventy-five individuals were randomly assigned to one of four treatment groups, but four individuals dropped out before drug administration. Two individuals in the 400 mg group had increased baseline HIA titre >1:8 and were excluded from efficacy analysis, so that the efficacy evaluable study population was 69. This group consisted of 59% men and 41% women; the age range was 18–43 years, with a median age of 21 years.

The proportion of individuals who demonstrated viral shedding was 58% in the placebo group (Table 3). This frequency did not differ significantly from those observed in the peramivir 50 mg (61%), 200 mg (37%), and 400 mg (31%) dose groups. However, the frequency and duration of viral shedding, viral AUC values, and the overall frequencies of infection tended to be lower in the two higher dose groups compared with placebo (Table 3). The peak viral titre was reduced by approximately 1 log_{10} and the mean duration of viral shedding by about 1 day in the peramivir 200 mg and 400 mg groups compared with placebo. No obvious differences in virological markers were noted between the 200 mg and 400 mg dose groups. The frequencies of cough and URI, nasal mucus discharge weight, and total symptoms scores did not

Table 2. Antiviral and clinical effects of early peramivir treatment in experimental B/Yamagata/16/88 influenza virus infection

Outcomes	800 mg qd (n=18)	800 mg qd/400 mg qd (n=18)	Placebo (n=19)
Infected individuals, *n* (%)	15 (83)	13 (72)	18 (95)
Shedding virus, *n* (%)	8 (42)	3 (17)	8 (42)
Viral titre AUC*† mean (SE)	2.7 (1.0; P=0.004)	3.5 (1.1 P=0.026)	6.7 (0.9)
Peak viral titre, $TCID_{50}$/ml (SE)	1.9 (0.4)	2.0 (0.4)	2.6 (0.3)
Duration of viral shedding, mean days (SD)	1.6 (0.3; P=0.026)	2.3 (0.5)	3.2 (0.5)
Cumulative nasal mucus weight, mean g (SE)*	20.5 (7.6)§	11.8 (7.5)	29.3 (7.6)¶
Individuals with fever, *n* (%)	0	0	0
Composite symptom score‡, mean (SE)	8.3 (1.6)	5.0 (1.7)	7.3 (1.4)

*Based on area under the curve $(AUC)_{days\ 1-7}$. †Log_{10} 50% tissue culture-infectious doses per ml x day. ‡Based on $AUC_{days\ 1-7}$. §n=13 due to missing data. ¶n=14 due to missing data. SD, standard deviation; SE, standard error; qd, once daily.

Table 3. Antiviral effects of peramivir prophylaxis in experimental A/Texas/36/91(H1N1) influenza infection

Outcomes	50 mg qd (*n*=18)	200 mg qd (*n*=19)	400 mg qd (*n*=13)	Placebo (*n*=19)
Infected individuals, *n* (%)	16 (89)	8 (42; *P*=0.050)	5 (38; *P*=0.052)	14 (74)
Shedding virus, *n* (%)	11 (61)	7 (37)	4 (31)	19 (58)
Viral titre AUC*† mean (SE)	6.7 (1.5)	2.4 (1.4)	2.5 (1.7)	5.2 (1.4)
Peak viral titre, $TCID_{50}$/ml (SE)	2.6 (0.5)	1.1 (0.5)	0.9 (0.6)	2.1 (0.5)
Duration of viral shedding, mean days (SD)	2.9 (0.6)	1.4 (0.5)	1.4 (0.6)	2.6 (0.6)

*Based on area under the curve $(AUC)_{days\ 2\text{–}8}$. †Log_{10} 50% tissue culture-infectious doses per ml × day. SD, standard deviation; SE, standard error; qd, once daily.

Table 4. Antiviral effects of peramivir prophylaxis in experimental B/Yamagta/16/88 influenza inoculation

Outcomes	200 mg qd (*n*=17)	400 mg qd (*n*=17)	800 mg qd (*n*=18)	Placebo (n=20)
Infected individuals, *n* (%)	13 (76)	16 (94)	15 (83)	18 (90)
Shedding virus, *n* (%)	7 (41)	6 (35)	9 (47)	11 (55)
Viral titre AUC*† mean (SE)	2.6 (1.5)	3.4 (1.5)	2.6 (1.4)	5.3 (1.4)
Peak viral titre, $TCID_{50}$/ml (SE)	1.2 (0.4)	1.0 (0.4)	1.1 (0.4)	1.8 (0.4)
Duration of viral shedding, mean days (SD)	1.9 (0.7)	1.8 (0.7)	1.8 (0.5)	2.7 (0.6)

*Based on area under the curve $(AUC)_{days\ 2\text{–}9}$. †Log_{10} median tissue culture-infective dose per ml x day. SD, standard deviation; SE, standard error; qd, once daily.

differ significantly in the higher dose treatment groups compared with placebo (data not shown).

Prophylaxis – influenza B study

Eighty-eight individuals were enrolled in the study, but due to withdrawals before inoculation or drug dosing, 73 were randomized and received at least one dose of the study drug. Seventy-two individuals completed the course of drug administration. The group consisted of 62% men and 38% women; the age range was 18–47 years, with a median age of 21 years.

The proportion of individuals with viral shedding was 55% in the placebo group, and no significant reductions were noted in the peramivir 200 mg (41%), 400 mg (35%), and 800 mg (47%) dose groups. The mean duration of viral shedding was about 1 day shorter in each of the peramivir groups, without an obvious dose-effect, and viral titre AUC values about one-half of that in the placebo group (Table 4). Illness measures including symptoms scores and nasal mucus weights were low and did not differ among treatment and placebo groups (data not shown).

Safety

The incidence of adverse events during the studies was low and similar between active and placebo groups (Table 5). The most commonly reported adverse events were nausea and headache; these were usually mild in severity. There were seven adverse events (headache, nausea, vomiting, toothache, herpes simplex reactivation, rash and sinusitis) reported of marked severity reported by four individuals in peramivir recipients. Of these, only rash was considered to be likely related to the study drug. One report of gastritis in the 400 mg once daily treatment group (influenza A treatment study) was considered to have a probable relationship to the study drug. There were no significant changes in laboratory values from baseline throughout the studies (data not shown).

Two individuals were withdrawn from the study due to adverse events. One subject in the 800 mg group developed a rash likely related to the study drug, and another subject in the 400 mg group developed a UTI that resolved with antibiotic treatment. One serious adverse event was reported after a subject completed the study. A 21 year old black male developed a dilated cardiomyopathy, which resolved with angiotensin-converting enzyme-inhibitor treatment. As described elsewhere [19], this event was considered possibly related to the experimental influenza B infection but very unlikely related to the study drug. He was clinically stable with low normal cardiac output on echocardiography when last seen in 2005, nearly five years after the event.

Peramivir blood concentrations

Peramivir was rapidly absorbed following oral administration. In the influenza B prophylaxis study, plasma

Table 5. Pooled findings for adverse events in peramivir and placebo recipients – number (%) of individuals with treatment-emergent adverse events with an incidence ≥3%*

Body system/preferred term	Peramivir (*n*=225)	Placebo (*n*=78)
Any adverse event	64 (28.4)	29 (37.2)
Body as a whole-general disorders	10 (4.4)	4 (5.1)
Back pain	6 (2.7)	3 (3.8)
Fatigue	2 (0.9)	1 (1.3)
Pain	2 (0.4)	0
Central and peripheral nervous system disorders	15 (6.7)	6 (7.7)
Dizziness	3 (1.3)	2 (2.6)
Headache	11 (4.8)	4 (5.1)
Migraine	1 (0.4)	0
Gastrointestinal system disorders	25 (11.1)	9 (11.5)
Abdominal pain	7 (3.1)	2 (2.6)
Diarrhea	6 (2.7)	2 (2.6)
Nausea	9 (4.0)	3 (3.8)
Stomatitis ulcerative	1 (0.4)	2 (2.6)
Vomiting	7 (3.1)	1 (1.3)
Tooth disorder	3 (1.3)	0
Respiratory system disorders	8 (3.5)	7 (9.0)
Coughing	2 (0.9)	1 (1.3)
Pharyngitis	3 (1.3)	3 (3.8)
Rhinitis	2 (0.9)	3 (3.8)
Sinusitis	1 (0.4)	1 (1.3)
Skin and appendages disorders	4 (2.2)	0
Acne	2 (0.9)	0
Rash	1 (0.4)	0
Folliculitis	1 (0.4)	0
Reproductive disorders, female†	2 (0.9)	3 (3.8)
Dysmenorrhea	2 (0.9)	3 (3.8)

*Patients reporting individual symptom within each category may exceed total number of patients reporting events for that category due to some patients reporting multiple events. †Based on 93 and 34 women treated with peramivir and placebo, respectively.

levels began to rise within one hour of oral administration and achieved maximal concentration in 2.5 h (Figure 3). Peak plasma concentrations were approximately proportional to the dose but averaged only about 50, 100 and 200 ng/ml at doses of 200 mg, 400 mg and 800 mg, respectively. In comparison, the mean C_{max} of peramivir for the 400 mg dose was 130 ng/ml in the influenza A treatment study and was 171-193 ng/ml for the 800 mg dose in the influenza B treatment study after the first dose. Plasma trough levels were generally stable on days 4–6 of administration, a finding that suggested that steady-state levels were reached after the third day of administration. (Figure 4). The trough levels were also proportional to dose and averaged approximately 10, 20 and 40 ng/ml for daily doses of 200, 400 and 800 mg, respectively, at steady-state.

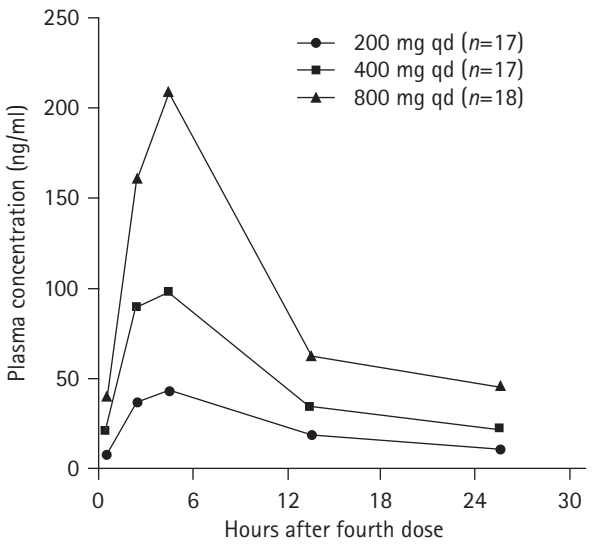
Susceptibility

In all four studies the last isolate from those shedding virus for at least three days were tested for susceptibility to peramivir. No significant increase in IC_{50} was found in any group. Among 52 isolates from peramivir recipients in the influenza A studies, the IC_{50} ranged from 0.25–0.6 nM, with mean values of 0.40–0.50 nM in individual studies (note: 1 nM equals 0.33 ng/ml). For 41 isolates from peramivir recipients in the influenza B studies, the IC_{50} values ranged from 1.1–1.95 nM, with mean values of 1.36–1.56 nM in individual studies. The challenge virus IC_{50} values were similar and ranged from 0.4 – 0.6 nM for influenza A/Texas/36/91, with a mean value of 0.49 nM in different assays, and 1.1–1.75 nM for influenza B/Yamagata/88 with a mean value of 1.45 nM.

Discussion

These studies demonstrate that the novel neuraminidase inhibitor peramivir exerts significant antiviral effects following oral administration in experimentally induced human influenza virus infections. The treatment studies demonstrated that the drug has

Figure 3. Plasma concentrations of peramivir before and following oral administration of the fourth dose (hour 0) in the influenza B prophylaxis study



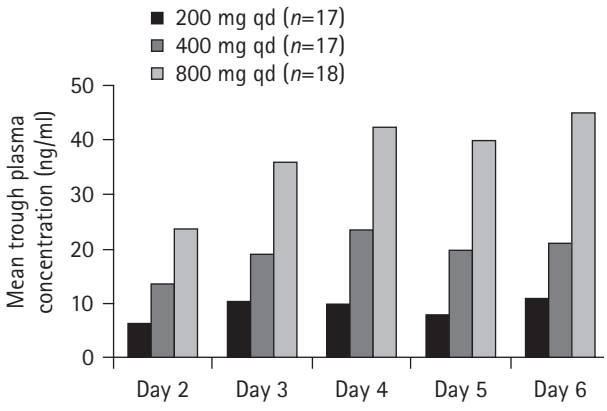
significant dose-related antiviral activity when used early in both influenza A and B infections and that once-daily dosing was inhibitory for viral replication. However, the prophylaxis studies found only modest, non-significant reductions in measures of viral replication. In comparison to earlier studies of oral oseltamivir in experimental human influenza A and B virus infections , oral peramivir generally exerted less robust antiviral effects. For example, in contrast to the current studies, oseltamivir prophylaxis at doses of 100 mg once or twice daily completely prevented recovery of influenza virus from inoculated volunteers and significantly reduced the overall incidence of infection following influenza A inoculation [16].

Although we observed trends towards reductions in some secondary clinical endpoints, illness incidence and other clinical measures were lower in the placebo groups than in previous studies of other NAIs. For example, in similar studies of zanamivir [15] and oseltamivir [16] 38% and 31% of placebo-treated, influenza A-infected individuals, respectively, developed fever subsequent to inoculation, compared with only 5 of 32 (15%) infected placebo individuals in the present influenza A studies. Consequently, we did not observe the reductions in symptom scores and nasal mucus weights observed in similarly conducted studies of zanamivir and oseltamivir. Although we could not compare the two drugs directly, our observations would predict that these doses of oral peramivir would not be as effective as oral oseltamivir in natural influenza. In this regard, an unpublished Phase III found that oral peramivir exerted antiviral effects but failed to significantly reduce the primary endpoint time of resolution of illness in uncomplicated influenza in adults, unlike earlier findings with oseltamivir or zanamivir treatment [20–22]. This led its sponsor to stop further clinical development of oral peramivir.

In addition to the mild nature of the experimentally induced illness observed, one reason for the lack of more robust antiviral and clinical effects in these studies is the human pharmacology of oral peramivir. The limited pharmacokinetic data collected in these studies are consistent with those seen in unpublished Phase I studies and indicate that peramivir has low oral bioavailability in humans. In comparison to levels of oseltamivir carboxylate observed after 75 (or 100 mg) doses [23], our observed blood levels were substantially lower despite use of higher doses. The peak peramivir plasma levels of ~100 ng/ml (400 mg dose) and ~200 ng/ml (800 mg dose) seen after four doses are substantially lower than the mean peak oseltamivir carboxylate concentrations of ~250–310 ng/ml observed after a single 100 mg dose [24] or of ~400–440 ng/ml after multiple 75 mg doses [25]. Similarly, the trough oseltamivir carboxylate concentrations with repeated doses of 100 mg (220–230 ng/ml) [24] greatly exceed the trough concentrations of peramivir at steady-state (~40–50 ng/ml at 800 mg doses). These differences indicate that the oral bioavailability of peramivir is much lower than the 80% value reported for oseltamivir and is probably less than 10%. Furthermore, the more prolonged plasma elimination half-life of peramivir, relative to oseltamivir, indicates that 3–4 days are required to reach steady-state levels and maximal antiviral efficacy. These factors could account for the lower prophylactic efficacy observed in these studies compared with earlier ones of oseltamivir. Further studies that use more sustained dosing prior to viral exposure would be required to test this possibility. However, the excellent antiviral activity of this compound, including highly pathogenic avian influenza viruses [10–12,26], low oral bioavailability, and prolonged plasma half-life suggest that a parenteral formulation could offer the potential for an effective treatment in humans. Of note, previous studies determined that intravenous zanamivir was highly active in experimental human influenza A virus infection [27], and parenteral formulations of peramivir are planned to enter clinical testing in the near future.

The drug was generally well tolerated with a low incidence of adverse events, the most common being nausea or headache. Most adverse events were mild to moderate severity. One serious adverse event (the development of cardiomyopathy) occurred in the 200 mg per day treatment group. This event was very unlikely to be related to the study drug but possibly could be

Figure 4. Mean trough plasma concentrations of peramivir determined 24 h after the last dosing interval in the influenza B prophylaxis study



linked to the experimental infection as similar events have been described in natural influenza infections [19]. Of note, oral peramivir was not associated with the gastrointestinal side effects (nausea and emesis) that are relatively common with oseltamivir.

The need for novel antivirals for influenza remains despite two active neuraminidase inhibitors already approved for use. The inhalation mode of delivery remains a challenge with zanamivir, in part because of concerns regarding bronchospam and because of difficulties in delivery, particularly in the elderly [28]. Importantly, influenza isolates with *in vitro* resistance have emerged during clinical use of oseltamivir [8,29,30] and rarely with zanamivir [7]. Some of these variants remain at least partially susceptible to peramivir [9,10]. In the current study testing of post-inoculation viral isolates by neuraminidase inhibition assay did not demonstrate any evidence for resistance emergence due to neuraminidase changes. In contrast, when oseltamivir was used for early treatment of experimental influenza A/Texas/36/91/H1N1 infection two of 54 volunteers had the emergence of viruses with neuraminidase mutations (His274Tyr) conferring high-level resistance to oseltamivir [29]. This resistant variant has been described in 16% of influenza A (H1N1)-infected children treated with oseltamivir [30]. However, peramivir-resistant variants of influenza have been selected [31] or generated [32] in the laboratory and some neuraminidase mutations confer resistance across the NAI class [33]. It remains to be determined whether the frequency of resistance emergence might be lower with peramivir than oseltamivir during clinical use.

In summary, peramivir demonstrated significant antiviral activity in experimentally induced human influenza A and B infections when used once daily. Prophylaxis studies indicated minimal protection against viral infection but may have been compromised by suboptimal dosing regimens. The low oral bioavailability of peramivir suggests that alternative formulations or parenteral administration warrant study.

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