Oral oseltamivir in human experimental influenza B infection

Frederick G Hayden[1], Lance Jennings[2], Richard Robson[3], Gilbert Schiff[4], Helen Jackson[5], Brinderjeet Rana[5], Graham McClelland[5], David Ipe[6], Noel Roberts[5] and Penelope Ward[5]*

[1]University of Virginia School of Medicine, Department of Internal Medicine, Charlottesville, Va., USA
[2]Canterbury Health Laboratories, Christchurch, New Zealand
[3]Department of Nephrology, Christchurch Hospital, Christchurch, New Zealand
[4]The Children's Hospital Medical Center, Cincinnati, Ohio, USA
[5]Roche Global Development, Welwyn, UK
[6]Roche Global Development, Palo Alto, Calif., USA

*Corresponding author: Tel: +1 804 924 5059; Fax: +1 804 924 9065; E-mail: fgh@virginia.edu

Oseltamivir is the prodrug of Ro64-0802 (GS4071), a potent and selective inhibitor of influenza A and B virus neuraminidases. Three randomized, double-blind, placebo-controlled, parallel-group studies evaluated oral oseltamivir for early treatment (75 or 150 mg twice daily for 5 days) or prevention (75 mg once or twice daily for 7 days) of experimental influenza B virus infection in healthy susceptible adults. Treatment study A (*n*=60) demonstrated similar trends to treatment study B (*n*=117), in which 75 mg doses of oseltamivir introduced 24 h after inoculation reduced median area under curve (AUC) virus titre (oseltamivir, 22.7; placebo, 131.1 $\log_{10}$ $TCID_{50}$ × h/ml; *P*=0.002) and duration of viral shedding (oseltamivir, 23.9 h; placebo, 95.8 h; *P*=0.0005). In prevention study C (*n*=58), oseltamivir did not reduce infection rates (85 versus 84%) but significantly reduced median AUC virus titre (10.0 versus 66.9 $\log_{10}$ $TCID_{50}$ × h/ml; *P*=0.03) and duration of viral shedding (36 versus 84 h; *P*=0.03) compared with placebo. Oseltamivir was well tolerated. No emergence of drug-resistant variants was detected by testing last-day isolates (*n*=112) in neuraminidase inhibition assays. These results indicate that oseltamivir has significant antiviral activity in experimental human influenza B virus infection when used for prophylaxis or early treatment.

Introduction

Influenza B outbreaks occur approximately every 2–3 years and are responsible for considerable morbidity and sometimes excess mortality, despite inclusion of influenza B in current vaccines [1–4]. Influenza B viruses circulate worldwide and in the 1998/1999 influenza season accounted for 23% of all influenza isolates detected in the USA [5]. Furthermore, mixed outbreaks can often occur in which influenza A and B viruses sequentially infect the population [6,7]. Infection rates can be high, particularly in children and sometimes in adults living in closed environments, where influenza B adult attack rates of up to 80% have been reported [7–11].

Antiviral agents offer a rational option for influenza management, but the currently available M2 inhibitors amantadine and rimantadine are ineffective against influenza B viruses. Recently, there has been considerable interest in developing antivirals that target the influenza neuraminidase enzyme [12]. This surface glycoprotein cleaves terminal sialic residues, enabling the release of virus from infected cells and preventing viral aggregation, and is therefore essential for viral replication. As the neuraminidase active site is highly conserved in all influenza virus strains [13,14], neuraminidase inhibitors should be active against all influenza A and B strains. Zanamivir was the first clinically effective neuraminidase inhibitor, but it has low oral bioavailability and is consequently administered topically by inhalation. Influenza virus replicates throughout the upper and lower respiratory tract [11] and in rare cases can be found at extrapulmonary sites [15–18]. Consequently, the availability of an orally administered drug that distributes to all virus-infected sites would be convenient and perhaps clinically advantageous, although no direct comparisons of efficacy have been made between oral and inhaled neuraminidase inhibitors in humans.

Oseltamivir (Ro64-0796, GS4104) is an oral prodrug and, after absorption from the gastrointestinal system, is rapidly converted to its active carboxylate metabolite (Ro64-0802, GS4071) [19]. Ro64-0802 is a potent and selective inhibitor of both influenza A and

B viruses *in vitro* and in experimentally infected animals [20–22]. Oseltamivir has previously been shown to be effective in the treatment and prevention of experimental and natural influenza A virus infection in humans [23–26]. The pivotal efficacy trials of oseltamivir in subjects with naturally acquired influenza infection took place during the Northern Hemisphere winter of 1997–1998, when only 2.6% of 887 influenza-infected subjects had laboratory evidence of infection with influenza B virus [23,26]. Consequently, we have conducted three studies to investigate the efficacy and tolerability of oseltamivir for treatment and prevention of experimental human influenza B infection.

Materials and Methods

Study design

Three randomized, double-blind, placebo-controlled, parallel-group studies were conducted to evaluate the efficacy and tolerability of oseltamivir treatment (studies A and B) and prophylaxis (study C) in volunteers with experimental influenza B virus infection. Studies A and C were single-centre studies, while study B was a multi-centre study conducted in the USA (two centres), New Zealand (one centre) and the UK (one centre). The studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. All three studies had local ethics committee approval, and the volunteers provided written informed consent. The overall trial designs are outlined in Figure 1.

Subjects

Healthy adult volunteers aged 18–65 years and of normal body weight were recruited. Eligible subjects were required to be susceptible to viral challenge, as defined by influenza B/Yamagata/16/88 haemagglutination inhibition (HAI) antibody titres of <1:10 for study A, and ≤1:8 for studies B and C. Subjects with chronic disease, those taking corticosteroids or immunosuppressive drugs or those who were pregnant, were excluded.

Infection and drug administration

Subjects were inoculated by dropper into each nostril with approximately 0.25 ml containing 10^7 tissue culture infectious doses ($TCID_{50}$) per ml of a safety-tested pool of influenza B/Yamagata/16/88 (National Institutes of Health, Bethesda, Md., USA) virus, either 24 h before (studies A and B) or 24 h after (study C) study drug initiation. Treatment regimens for the three studies are shown in Figure 1. Study drug was administered in a fasted state in studies A and B and after food in study C.

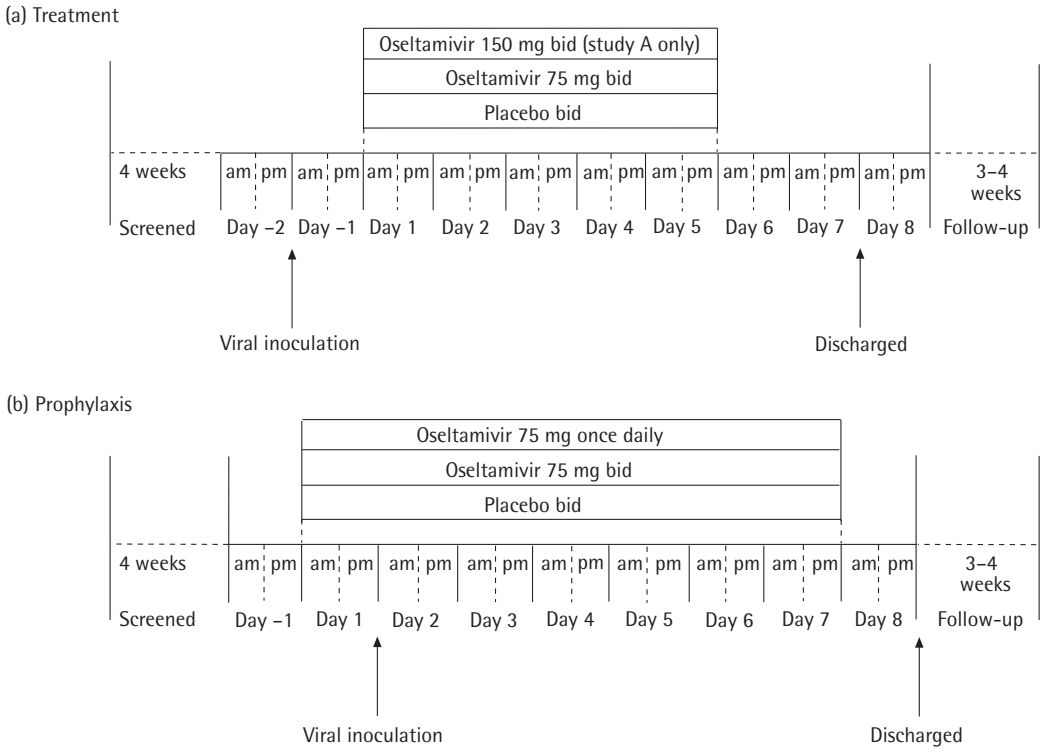
Acetaminophen (paracetamol) was supplied for relief of fever and discomfort and its use was documented. Drugs known to interfere with drug metabolism, gut motility or renal excretion were not permitted; other medications required for the relief of disease symptoms or other medical conditions arising were permitted at the discretion of the investigator.

Clinical and virological monitoring

Prior to inoculation, nasal washings were performed for influenza virus cultures as previously described [24] and 12-h nasal mucus weights were measured. Nasal washings were repeated before drug administration, every 12 h on days 1–3 (and from day 2 until the morning of day 6 in study C), and every 24 h thereafter until day 8 for recovery of virus and determination of viral titres. Nasal washes were performed by instillation of 5 ml sterile balanced salt solution into each nostril in turn with the patient's head tilted backwards. Patients were asked to remain in this position for 10–15 s while making hard 'K' sounds without swallowing. Saline, mucus and cell debris were gently expelled through the nose into a clean container when the head was tilted forwards. The total volume of nasal wash recovered per patient was typically 6–8 ml. Containers of nasal washings were mixed by pipetting with viral transport medium (4:1 v/v), placed on wet ice and processed (aliquoted, 1 ml), and cryopreserved (–70°C) within 15 h of sample collection from the patient. A portion of one aliquot was inoculated immediately onto confluent monolayers of Madin Darby canine kidney (MDCK) cells to detect infection with the challenge virus (without prior cryopreservation). The remaining cryopreserved aliquots were used for viral titration (if aliquot 1 was positive for influenza virus) and expansion of the primary isolate to test viral neuraminidase inhibition *in vitro*.

Subjects recorded oral temperature and completed symptom assessment scores 12-hourly at baseline through day 8. Absence/presence and severity of 14 symptoms (nasal stuffiness, earache/pressure, runny nose, sore throat, cough, sneezing, breathing difficulty, muscle ache, fatigue, headache, feverish feelings, hoarseness, chest discomfort and overall discomfort) were recorded on a four-point scale (absent–severe). Assay of strain-specific influenza B HAI antibody titre was performed on paired sera collected before inoculation and follow-up between days 21–28. The susceptibility of viral isolates from studies B and C were assessed by the sensitivity of the viral neuraminidase to inhibition by oseltamivir carboxylate. The assay used was based on that of Potier *et al.* [27], but used a much higher substrate concentration to enhance assay sensitivity. This gives rise to artificially high IC_{50} values, for example, for B/Yamagata

Figure 1. Overall trial design for (a) treatment studies A and B and (b) prophylaxis study C



bid, twice daily.

neuraminidase, IC_{50} in this assay is 65.2 nM compared with a K_i of 1.9 nM.

Efficacy endpoints

Influenza infection was defined by virus recovery from samples taken ≥24 h after inoculation or a ≥4-fold increase in serum strain-specific HAI antibody titre compared with baseline. The primary efficacy endpoint for treatment studies A and B was the change in the viral burden, calculated as the area under the virus titre versus time curve (AUC). The primary efficacy parameter for prevention study C was the proportion of patients with influenza infection.

Other end-points included peak virus titre, the duration of viral shedding [defined as the time from treatment initiation (studies A and B) or inoculation (study C), to the first negative nasal wash with no subsequent positive washes], and the effect on influenza symptoms. Composite symptom scores were calculated by totalling the scores of a subset of the 14 assessed symptoms. This subset, comparable to that collected in treatment studies in natural influenza [23,26], comprised the individual scores for the symptoms of feverishness, headache, myalgia, sore throat, cough and feeling bad, in addition to nasal symptoms (defined as the maximum score for nasal stuffiness or runny nose, whichever was the greater). The time to alleviation of composite symptoms was defined as the time from the initiation of treatment to the first time that each was absent or had a maximum severity of 1, with no subsequent severity grades >1. In addition, the AUC for symptom scores over time was calculated from the time of initiating drug administration.

Adverse events were recorded throughout the study. Vital sign measurements were performed 12-hourly throughout the study and at follow-up. Standard clinical laboratory tests (haematology, biochemistry and urinalysis) were performed before viral inoculation and during treatment.

Data analysis

The efficacy analyses included randomized, infected (studies A and B only), sero-susceptible subjects with no major protocol violations who had received at least one dose of study treatment. In studies A and C, data from the oseltamivir dose groups were pooled for comparison with placebo. The virus titre AUCs, peak virus titres and composite symptom score AUCs were compared between groups using a Wilcoxon rank sum test. Lehmann estimates were used to calculate the 95% confidence interval (CI) for the difference between the medians for the AUC viral titre. Duration of viral shedding and composite symptoms were compared between groups using a Mantel–Haenszel test with generalized Gehan–Wilcoxon weighting. A sub-analysis was performed on the duration of viral

shedding in the subset of patients in study A who exhibited viral shedding at baseline. In study B, the viral titre AUCs and composite symptom AUCs were compared between the oseltamivir and placebo groups using an extended Wilcoxon rank sum test stratified by centre. Lehmann estimates were used to calculate 95% CI for the difference between the unstratified medians for the AUC viral titres. All statistical tests in study B were stratified by centre. In study C, the proportion of subjects with infection was compared between the placebo and active treatment populations using a two-sided Fisher's exact test.

The tolerability/safety analysis included all randomized patients. All efficacy analyses were performed using SAS 6.12 software (SAS Institute, Cary, N.C., USA).

Sample size calculations were performed using nQuery Version 2.0 computer software (Dixon Associates, Los Angeles, Calif., USA). For study A, a *t*-test with a two-sided 5% significance level indicated that 16 infected subjects per group were required to detect a difference in the virus titre AUC between the pooled oseltamivir and placebo groups of 105.6 $\log_{10}$ $TCID_{50}$×h/ml (standard deviation 120 $\log_{10}$ $TCID_{50}$×h/ml) with 80% power. For study B, the same methods were used to calculate the power to detect a range of clinically relevant differences in the viral titre AUC between oseltamivir and placebo (120–168 $\log_{10}$ $TCID_{50}$×h/ml) assuming a range of standard deviations (192–288 $\log_{10}$ $TCID_{50}$×h/ml). A sample size of 30 placebo recipients and 60 oseltamivir recipients provided power that ranged from 45 to 97% for all the above combinations of standard deviations and expected differences. For Study C, a two-group continuity corrected χ^2 test with a two-sided 5% significance level had 88% power to detect a difference between the placebo infection proportion of 0.67 and the pooled active group infection proportion of 0.22 when sample sizes were 20 and 40, respectively.

Results

Subjects and infection rates

Study A. Sixty subjects were screened and randomized 1:1:1 to treatment with oseltamivir 75 mg twice daily (*n*=20), oseltamivir 150 mg (*n*=20) or placebo (*n*=20), and all completed the study. Twenty-one subjects (seven in each group) were excluded from the efficacy analysis because they did not show influenza B infection and/or because re-testing of baseline antibody titre values showed them to be seropositive (titre ≥1:10) before challenge. Thus, the efficacy evaluable population consisted of 39 subjects (13 in each group).

Study B. A total of 117 subjects were screened and randomized 2:1 to treatment with oseltamivir 75 mg (*n*=78) or placebo (*n*=39), and all completed the study. Thirty-one subjects (21 oseltamivir, 10 placebo) were excluded from the efficacy analysis because they did not show influenza B infection or because they were seropositive at baseline (HAI titre >1:8). The efficacy evaluable population consisted of 86 subjects (57 oseltamivir, 29 placebo). The proportions of subjects successfully infected were similar between the oseltamivir and placebo groups (*P*=0.8) and between treatment centres.

Study C. Fifty-nine subjects were enrolled and randomized 1:1:1 to treatment, but one subject withdrew from the study prior to receiving treatment. Therefore, 58 subjects received oseltamivir 75 mg once daily (*n*=19), 75 mg twice daily (*n*=20) or placebo (*n*=19). All 58 subjects completed the study and comprised the intent-to-treat (ITT) population.

The demographic characteristics of subjects with regard to age, sex, weight, height and race were similar across treatment groups in all three studies.

Efficacy

For the two treatment studies (A and B) efficacy analysis was restricted to those with laboratory documented infection. Few febrile illnesses or upper respiratory symptoms were noted in all three studies (Table 1). The frequency of acetaminophen use during study drug administration was low and similar between oseltamivir and placebo recipients (study A: oseltamivir, 150 mg, 20%; oseltamivir, 75 mg, 7%; placebo, 6%; study B: oseltamivir, 75 mg, 26%; placebo, 28%; study C: oseltamivir, 75 mg twice daily, 20%; oseltamivir, 75 mg once daily, 27%; placebo 21%).

Study A. In treatment study A, no significant differences in virological outcomes were seen between the two oseltamivir doses (Table 1), and they were combined for analysis. In the subset of sero-susceptible subjects, the median AUC virus titre in the pooled oseltamivir group (75 or 150 mg twice daily) was reduced by 96% compared with that of placebo (5.9 versus 149.7 $\log_{10}$ $TCID_{50}$ × h/ml; *P*=0.09) and median peak viral titre was reduced by 2 $\log_{10}$ (0.3 versus 2.3 $\log_{10}$ $TCID_{50}$/ml; *P*=0.08). The proportion of subjects with detectable viral shedding was 69% in the oseltamivir 75 mg group, 62% in the 150 mg group, and 85% in the placebo group. Oseltamivir treatment reduced the duration of viral shedding by approximately 78 h compared with placebo (median, 18 h versus 96 h; *P*=0.04).

The median duration of illness (composite symptoms) was reduced by 82% in the pooled oseltamivir group compared with placebo (23 versus 131 h; *P*=0.08), and symptom score AUC was reduced by

Table 1. Summary of clinical and virological measures in adults experimentally infected with influenza B/Yamagata/16/88 and given oseltamivir or placebo for prevention or treatment

Treatment group	No. ≥4-fold serological rise (%)*	No. infected (%)	No. shedding virus (%)	GM-fold change in HAI titre	No. URI (%)	No. fever (%)	Comp. symptom score AUC (score x h) (IQ range)	Median cum. nasal mucus weight (g) (IQ range)	Median viral titre AUC (log_{10} $TCID_{50}$ x h/ml) (IQ range)	Median peak viral titre log_{10} $TCID_{50}$/ml (IQ range)
Study A treatment										
Oseltamivir 75 mg bid	9/13 (69)	15/20 (75)	9/13 (69)	5.2	7/13 (54)	0/13 (0)	202.1 (95.6–280.5)	3.3 (0.7–7.0)	6.0 (0.0–52.0)	0.5 (0.0–1.5)
Oseltamivir 150 mg bid	12/13 (92)	15/20 (75)	8/13 (62)	7.2	7/13 (54)	0/13 (0)	279.4 (150.0–371.6)	5.7 (0.0–10.2)	5.8 (0.0–85.2)	0.0 (0.0–2.8)
Placebo	10/13 (77)	16/20 (80)	11/13 (85)	5.5	9/13 (69)	0/13 (0)	311.0 (252.0–489.8)	9.0 (1.7–26.0)	149.7 (13.4–302.6)	2.3 (0.8–4.3)
Study B treatment										
Oseltamivir 75 mg bid	45/57 (79)	58/78 (74)	48/57 (84)	4.7	6/57 (11)	1/57 (2)	117.2 (65.5–194.9)	7.8 (2.1–11.6)	22.7 (8.9–58.9)	1.5 (0.8–2.3)
Placebo	22/29 (76)	30/39 (77)	26/29 (90)	5.6	7/29 (24)	1/29 (3)	184.1 (120.1–341.0)	9.9 (4.7–25.3)	131.1 (27.9–298.2)	2.8 (1.8–3.8)
Study C prophylaxis										
Oseltamivir 75 mg once daily	16/19 (84)	17/19 (89)	11/19 (58)	6.0	2/19 (11)	1/19 (5)	95.4 (36.0–185.0)	15.6 (8.3–22.5)	36.0 (0.0–173.2)	1.2 (0.0–2.8)
Oseltamivir 75 mg bid	13/20 (65)	16/20 (80)	11/20 (55)	4.1	0/20 (0)	0/20 (0)	90.0 (30.0–167.6)	10.0 (7.4–15.1)	10.0 (0.0–63.4)	0.8 (0.0–1.9)
Placebo	12/19 (63)	16/19 (84)	14/19 (74)	4.6	4/19 (21)	2/20 (10)	125.9 (30.0–298.1)	15.7 (11.1–23.9)	66.9 (0.0–324.7)	1.8 (0.0–4.5)

AUC, area under the curve; bid, twice daily; comp., composite; cum., cumulative; GM, geometric mean; IQ, inter-quartile; URI, upper respiratory tract infection; **n* seroconversion. The outcomes in the treatment studies (A and B) are listed only for those volunteers with laboratory-documented infection (virus shedding and/or seroconversion).

Figure 2. Median virus titre versus time in influenza B-infected, sero-susceptible subjects treated with oseltamivir 75 mg or placebo twice daily in study B

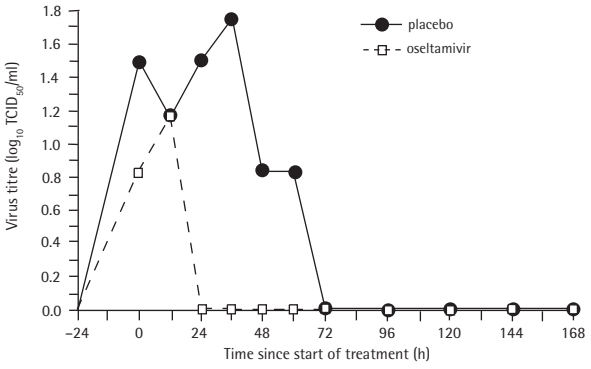
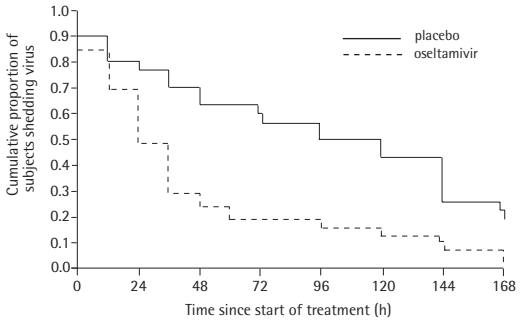


Figure 3. Proportion of influenza B-infected, sero-susceptible subjects shedding virus versus time, after treatment with oseltamivir 75 mg or placebo twice daily in study B



16% (261 versus 311 score × h; *P*=0.05).

Study B. Treatment with oseltamivir (75 mg twice daily) reduced the median AUC virus titre by 83% compared with placebo (22.7 versus 131.1 log_{10} $TCID_{50}$ × h/ml; *P*=0.002) (Figure 2). Peak virus titres occurred approximately 2–3 days after virus inoculation for those in the placebo group (Figure 2). As the viral titre data immediately prior to drug administration was slightly imbalanced, the primary end-point was adjusted for baseline viral titre. This *post hoc* analysis still resulted in a significant effect for oseltamivir (*P*=0.02). The median peak virus titre was reduced by 1.3 log_{10} $TCID_{50}$/ml in the oseltamivir group compared with placebo (1.5 versus 2.8 log_{10} $TCID_{50}$/ml) (Table 1). The median duration of viral shedding was reduced by approximately 72 h (75%) in the oseltamivir group compared with placebo (24 versus 96 h; *P*=0.0005) (Figure 3).

Oseltamivir reduced the median AUC for the composite symptom score by 36% compared with placebo (117 versus 184 score × h; *P*=0.07) (Table 1), and reduced the median AUC for total symptom score (226 versus 338 score × h; *P*=0.07). The median time to alleviation of composite symptoms was brief in both oseltamivir (0.0 h) and placebo (11.5 h) groups. The proportions of infected persons developing upper respiratory tract infections were only 11 and 24% in oseltamivir and placebo, respectively (Table 1).

Study C. In the prophylaxis study, no significant differences in virological outcomes were seen between the oseltamivir once and twice daily groups, so they were combined for analysis. A similar number of subjects were infected with influenza B (detected either by culture or a ≥4-fold increase in HAI antibody) in both the pooled oseltamivir (85%) and placebo (84%) groups. When influenza infection was defined as positive viral culture only, the proportion of subjects shedding virus was 24% lower in the pooled oseltamivir group compared with placebo (56 versus 74%; *P*=0.3).

The median viral AUC titre was 85% lower (10.0 versus 66.9 log_{10} $TCID_{50}$ × h/ml; *P*=0.03), and median peak viral titre was reduced by 1.0 log_{10} (0.8 versus 1.8 log_{10} $TCID_{50}$/ml; *P*=0.04) in subjects receiving oseltamivir compared with placebo. The duration of viral shedding was 48 h (57% reduction) shorter in oseltamivir compared with placebo recipients (36 versus 84 h; *P*=0.03). The composite symptom score AUCs were low and did not differ between the oseltamivir (96 score × h) and placebo (126 score × h) groups.

Safety and tolerability

There were no serious adverse events during the studies and no premature withdrawals due to adverse events. Oseltamivir had no clinically significant effects on clinical laboratory parameters or vital signs in any of the three studies (data not shown).

Oseltamivir was generally well tolerated. Transient adverse events among active drug recipients in the treatment studies were primarily nausea of mild-to-moderate intensity (study A: oseltamivir 75 mg 5/20, 150 mg 8/20, placebo 1/20; study B: oseltamivir 75 mg 8/78, placebo 3/39) and less often vomiting (study A: oseltamivir 75 mg 2/20, 150 mg 1/20, placebo 0/20; study B: oseltamivir 75 mg 4/78, placebo 1/39).

Few of the adverse events that were considered related to study treatment were reported during study C (1/19 receiving oseltamivir once daily, 3/20 receiving oseltamivir twice daily, and 1/19 receiving placebo). Transient mild nausea was reported by 3/20 twice daily oseltamivir recipients. There were no treatment-related adverse events during the off-treatment follow-up period.

Neuraminidase enzyme susceptibility of isolates

A total of 112 last-day isolates (71 oseltamivir, 41 placebo) from studies B and C were tested for sensitivity by neuraminidase inhibition assay to oseltamivir carboxylate. The challenge strain had a mean IC_{50} value of 65.2 nM (range, 40.9–93.6 nM) using this assay method. The sensitivities (mean and range) were very similar for isolates from both placebo and oseltamivir-treated groups and both were similar to the challenge virus. In study B the mean (range) IC_{50} values were 159 nM (range, 79–265 nM) for oseltamivir, 156 nM (range, 83–227 nM) for placebo. In study C the mean (range) IC_{50} values were 157 nM (range, 75–218 nM) for oseltamivir and 155 nM for placebo (range, 121–183 nM). Thus, none of the isolates showed reduced susceptibility resulting from exposure to oseltamivir carboxylate.

Discussion

Although influenza B virus is less common than influenza A virus, it is nevertheless an important contributor to the morbidity and mortality of influenza disease [11]. Our findings indicate that oral oseltamivir has significant antiviral efficacy in experimental human influenza B infection. When used for early treatment, beginning 24 h after virus inoculation, oseltamivir diminished viral titres, the duration of shedding, and hence overall viral burden. Antiviral effects were observed with once or twice daily prophylactic administration. The primary dose level used in the studies (75 mg) is the one approved for twice daily dosing for treatment of influenza in adults.

The human experimental influenza A virus infection model has proved to be a valid model in which to assess the efficacy of antivirals [28]. Oseltamivir was effective for prevention and early treatment in challenge studies with influenza A, which used a similar protocol to the present studies [24]. Subsequently, the drug has also proved effective in treatment and prevention of natural influenza A infection [23,25,26]. The antiviral effects demonstrated here predict that oseltamivir would be expected to be effective in the treatment and prevention of natural influenza B infection in humans, although further studies on natural influenza B illness are needed.

The results of study A were confounded by the inadvertent recruitment of seropositive subjects, owing to the initial use of an insufficiently sensitive assay. Previous studies that have recruited patients with protective levels of influenza antibody have also been compromised by the effect of residual immunity to the virus [28,29]. Inclusion of these subjects reduced the statistical power of the study and is likely to have accounted for the lack of statistical significance for most of the treatment effects. Nevertheless, in serosusceptible subjects, oseltamivir substantially reduced the virus titre AUC, the duration of viral shedding and peak viral titre, compared with placebo. Study B was a larger trial that focused on the 75 mg twice daily (marketed) dosage of oseltamivir. It confirmed that this oseltamivir dose significantly reduced multiple measures of viral replication.

Oseltamivir prophylaxis did not reduce infection rates determined by serology or qualitative virus recovery, but significantly reduced quantitative measures of viral replication (Table 1). A small study of intranasal zanamivir in experimental influenza B found no significant treatment effect on the proportion of subjects infected, although the number of subjects exhibiting virus shedding was reduced in zanamivir recipients [30]. In experimental human influenza, the relatively high inoculum of influenza B virus delivered directly to the nasal mucosa is not reflective of naturally acquired infection due to aerosol transmission of lower viral inocula. The antiviral results demonstrated in this study suggest a sufficient drug level would be achieved to reduce viral replication following a lower natural virus exposure. Similar prophylactic doses to those used in this study have already demonstrated significant prophylactic efficacy against naturally acquired influenza A [25], and a field study during the 1998–1999 winter demonstrated the protective efficacy of oseltamivir in the prevention of naturally acquired influenza illness in close contacts of a primary cases of both influenza A and B infection [Data on file, F Hoffmann-La Roche].

It is difficult to assess the effect of antivirals on the severity of influenza illness in human challenge studies because symptoms are typically milder in experimental influenza than in natural infection [24]. In particular, coughing and fever are very uncommon with this challenge pool of B/Yamagata virus, and coryzal illness is moderate. Nevertheless, both treatment studies showed that oseltamivir substantially reduced the symptom score AUC and the duration of clinical illness compared with placebo. These findings concur with the results of challenge studies on oseltamivir with influenza A [24].

Experience of drug-resistant variants is an important concern in influenza. At the end of the treatment period, virus isolates from studies B and C were assayed for reduced neuraminidase sensitivity to oseltamivir carboxylate. None of the isolates from oseltamivir recipients were found to have a reduced susceptibility compared with isolates from placebo recipients. Such results confirm data from clinical studies on oseltamivir, in which only 1.5% of influenza variants developed reduced neuraminidase sensitivity when oseltamivir was used for treating influenza in

immunocompetent adults [26]. In addition, serial passage of influenza A virus in the presence of the oseltamivir carboxylate *in vitro* produced variants with reduced susceptibility to the drug [31], but this approach has not yet succeeded in selecting resistant influenza B viruses. Importantly, viruses from both *in vitro* and clinical studies were considerably less infectious than the wild-type in animal models [31–33]. These observations indicate that emergence of clinically relevant oseltamivir carboxylate-resistant influenza B viruses is likely to be very uncommon during clinical use of oseltamivir.

Oseltamivir has highly selective effects on influenza neuraminidases [21]. In common with previous trials in healthy subjects [24] and in patients [23,25,26], the present studies showed oseltamivir to be well tolerated. Mild-to-moderate, transient gastrointestinal adverse events were observed in some oseltamivir recipients. However, very few gastrointestinal effects were seen in study C (in which oseltamivir was administered after food rather than after fasting), confirming previous findings that these effects may be minimized or even abolished by taking oseltamivir with food [24]. Importantly, no patients discontinued treatment during these studies because of adverse events. Oseltamivir administration did not significantly affect the distribution of convalescent serum HAI titres compared with placebo in any of the studies, suggesting that it does not impair the ability of the host to mount an immune response to the influenza virus.

In conclusion, oseltamivir significantly reduces viral replication when used for early treatment or prophylaxis of subjects experimentally infected with influenza B virus.

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