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Pharmacokinetics and Tolerability of Oseltamivir Combined with Probenecid▽†

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Oseltamivir is an inhibitor of influenza virus neuraminidase, which is approved for use for the treatment and prophylaxis of influenza A and B virus infections. In the event of an influenza pandemic, oseltamivir supplies may be limited; thus, alternative dosing strategies for oseltamivir prophylaxis should be explored. Healthy volunteers were randomized to a three-arm, open-label study and given 75 mg oral oseltamivir every 24 h (group 1), 75 mg oseltamivir every 48 h (q48h) combined with 500 mg probenecid four times a day (group 2), or 75 mg oseltamivir q48h combined with 500 mg probenecid twice a day (group 3) for 15 days. Pharmacokinetic data, obtained by noncompartmental methods, and safety data are reported. Forty-eight subjects completed the pharmacokinetic analysis. The study drugs were generally well tolerated, except for one case of reversible grade 4 thrombocytopenia in a subject in group 2. The calculated 90% confidence intervals (CIs) for the geometric mean ratios between groups 2 and 3 and group 1 were outside the bioequivalence criteria boundary (0.80 to 1.25) at 0.63 to 0.89 for group 2 versus group 1 and 0.57 to 0.90 for group 3 versus group 1. The steady-state apparent oral clearance of oseltamivir carboxylate was significantly less in groups 2 (7.4 liters/h; 90% CI, 6.08 to 8.71) and 3 (7.19 liters/h; 90% CI, 6.41 to 7.98) than in group 1 (9.75 liters/h; 90% CI, 6.91 to 12.60) ($P < 0.05$ for both comparisons by analysis of variance). The (arithmetic) mean concentration at 48 h for group 2 was not significantly different from the mean concentration at 24 h for group 1 (42 ± 76 and 81 ± 54 ng/ml, respectively; $P = 0.194$), but the mean concentration at 48 h for group 3 was significantly less than the mean concentration at 24 h for group 1 (23 ± 26 and 81 ± 54 ng/ml, respectively; $P = 0.012$). Alternate-day dosing of oseltamivir plus dosing with probenecid four times daily achieved trough oseltamivir carboxylate concentrations adequate for neuraminidase inhibition in vitro, and this combination should be studied further.

Pandemic influenza virus infection has the potential to cause significant morbidity and mortality in the United States and elsewhere (27). Avian influenza A virus (bird flu, H5N1 variant) has caused unprecedented disease in poultry in several Asian countries and has the potential to be the cause of human pandemic influenza virus infection (36). Several human cases that resulted from significant bird exposure and among which the mortality rate was significant have also been reported (37). Recent data now suggest that the pandemic influenza virus strain, which resulted in overwhelming mortality in the 1918 pandemic, was an avian influenza strain (30, 31).

Although the risk of avian influenza virus transmission to health care workers, immediate contacts, family members appears to be low, at least one fatal human case appears to have resulted from human-to-human transmission (4, 19, 33). Although one H5N1 vaccine has been approved, current standard influenza vaccines do not incorporate the H5N1 or pandemic virus variants. In addition, traditional antiviral agents such as amantidine and rimantidine do not have activity against H5N1 variants. Oseltamivir (Tamiflu), an ethyl ester prodrug of Ro 64-0802 (the active metabolite of oseltamivir, oseltamivir carboxylate), is a selective inhibitor of influenza virus neuraminidase, has activity against most H5N1 influenza virus strains, and is approved for use for both the treatment of and prophylaxis against influenza A and B virus infections (3, 14, 24).

The current recommendations for the treatment of influenza A and B virus infections with oseltamivir suggest a dose of 75 mg taken orally twice daily (BID) for 5 days at the onset of symptoms or on the laboratory confirmation of infection. Rec-

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ommendations for prophylaxis against influenza A virus infection for those people exposed to or at high risk for exposure suggest a dose of 75 mg orally taken once daily for up to 6 weeks. Although the supply of oseltamivir is adequate to meet the current expected needs during the influenza season, shortages of U.S. influenza vaccine supplies during a resultant influenza epidemic or an emergent avian influenza outbreak may result in the rapid use of the existing supplies of oseltamivir. In addition, oseltamivir has a shelf life of 5 years, so many governments have chosen to maintain stockpiles in the event of an emergency. Therefore, given the potential for an emerging pandemic of human infection with an avian or other influenza virus strain, the limited availability of an effective vaccine, and the potentially limited supply of an antiviral with known in vitro activity, studies are required to explore alternative oseltamivir dosing strategies for prophylaxis against pandemic or avian influenza virus.

Oseltamivir phosphate is readily absorbed and rapidly converted (half-life [$t_{1/2}$], 1 to 3 h) to its active carboxylate metabolite via hepatic esterases (oseltamivir [Tamiflu] package insert; Roche, 2008). At least 75% of an oral dose reaches the systemic circulation as oseltamivir carboxylate, while less than 5% of an oral dose reaches the systemic circulation as oseltamivir phosphate. Once it is formed, oseltamivir carboxylate is minimally bound (3%) to human plasma proteins. It has a $t_{1/2}$ of 6 to 10 h and is eliminated by the kidney by a first-order process that includes glomerular filtration and tubular secretion by an anionic transporter system (9).

Probenecid is a medication used for the treatment of gout. It inhibits renal tubular urate resorption and has been found to decrease the levels of excretion of several medications, probably through inhibition of organic ion transporter 1 in renal tubular cells. Several studies have been published indicating that probenecid given one to three times daily at doses ranging from 250 to 1,000 mg can result in significant increases in the plasma concentrations of other approved medications (7, 15, 17, 22, 23). Probenecid demonstrates dose-dependent pharmacokinetics and nonlinear elimination (10, 35).

The coadministration of a single 150-mg dose of oseltamivir and probenecid (500 mg orally four times a day [QID] for 4 days) resulted in steady-state oseltamivir carboxylate concentrations that were 2.5-fold higher than those achieved with oseltamivir administration alone (13). No other dosing strategies with probenecid in combination with oseltamivir have been investigated. The primary objectives of this study were (i) to assess the safety of different dosing regimens of oseltamivir plus probenecid and (ii) to characterize the pharmacokinetics of oseltamivir carboxylate in the different regimens.

MATERIALS AND METHODS

Study design. This was a multicenter, open-label, randomized, three-arm pharmacokinetic drug interaction study. The subjects were randomly assigned to one of three treatment strategies. Each treatment was administered orally, as follows: the subjects in group 1 received a single dose of 75 mg of oseltamivir taken orally every 24 h (q24h) for 15 days, the subjects in group 2 received a single dose of 75 mg of oseltamivir taken orally every 48 h (q48h) plus probenecid at 500 mg taken orally QID for 15 days, and the subjects in group 3 received a single dose of 75 mg of oseltamivir taken orally q48h plus probenecid 500 mg taken orally BID for 15 days.

Sample size and subjects. The study was approved by the institutional review boards of the respective centers participating in the study. Written informed consent was obtained from each subject prior to enrollment in the study. The target sample size was 48 subjects, with 24 subjects being <65 years of age and 24 subjects being ≥65 years of age. The sample size was calculated by using the variability in the oseltamivir carboxylate area under the concentration-time curve (AUC) reported in a previously conducted pharmacokinetic investigation (coefficient of variation, ≅20%) (oseltamivir [Tamiflu] package insert; Roche, 2008). With α equal to 0.05, a sample size of 48 (16 subjects per group) was calculated to provide an 85% power to determine a significant difference of 30% in oseltamivir carboxylate AUC values between the study groups (SYSTAT Software Inc., Richmond, CA). Sample size was also determined by using a standardized table for estimation of the sample size for average bioequivalence testing (34). With α equal to 0.05 and σ equal to 0.3, the use of 44 total subjects was deemed appropriate to provide a >80% power to test for average bioequivalence between multiple groups.

The subjects were allowed to take medications concomitantly, except for the following: acyclovir, allopurinol, penicillamine, clofibrate, rifampin, methotrexate, zidovudine, theophylline, dapsone, penicillins or cephalosporins, nonsteroidal anti-inflammatory medications, sulfonamides, oral hypoglycemics, barbiturates, and benzodiazepines. The subjects could not have active medical problems, a history of glucose-6-phosphate dehydrogenase deficiency, gout, significant blood dyscrasias, a history of hypersensitivity to sulfonamide drugs, kidney disease, kidney stones, poorly functioning kidneys, or active peptic ulcer disease or to have recently been exposed to an influenza virus. A medical history, physical examination, 24-h urine collection for the determination of creatinine clearance, clinical laboratory safety tests (serum chemistry and hematology), and urinalysis were performed at a screening visit. The subjects were excluded if any of the following laboratory conditions existed: hemoglobin concentrations of ≤10.0 g/dl for males and ≤9.0 g/dl for females, a platelet count of ≤75,000/μl, an absolute neutrophil count of ≤1,000/μl, serum aspartate aminotransferase and serum alanine aminotransferase levels more than 2.5 times the normal upper limit, any elevated serum uric acid level, serum creatinine levels >1.5 times the normal upper limit for subjects <65 years of age and any increase above the upper limit of normal for subjects ≥65 years of age, and a creatinine clearance of ≤50 ml/min. The subjects were monitored for adverse events throughout the study. The subjects were seen on days 0, 1, 4, 8, 14, 15, 16, 21, and 28. To assess dosing compliance (calculated as the number of doses taken divided by the number of scheduled doses), pill counts were performed on days 4, 8, and 14 and random whole-blood samples (5 ml) were collected for determination of probenecid, oseltamivir, and oseltamivir carboxylate concentrations at the baseline and 1, 4, and 8 days following the initiation of oseltamivir dosing. Blood samples were collected into Vacutainer tubes containing either sodium heparin (probenecid) or potassium EDTA (oseltamivir and oseltamivir carboxylate).

On day 15 following the initiation of oseltamivir treatment, blood samples (5 ml collected in a sodium heparin Vacutainer tube and 5 ml collected in a potassium EDTA Vacutainer tube) were collected immediately prior to the morning doses of oseltamivir and probenecid and at 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12, 18, 24, and 48 h afterward. All blood samples collected for pharmacokinetic analysis were collected within 10 min of the scheduled time. The actual times of collection were used to calculate the pharmacokinetic parameter values. Subjects were observed in the study unit at each clinical center for up to 24 h during this period of collection of samples for pharmacokinetic analysis. The oseltamivir and probenecid doses were administered by study unit personnel generally between 8 and 9 a.m. Follow-up examinations and blood and urine clinical laboratory safety tests were performed on days 1, 4, 8, and 14 of the dosing phase and 1 and 2 weeks after completion of the dosing phase of the study. The total volume of blood collected for the entire study was approximately 275 ml. After the baseline, creatinine clearance was calculated by using the formula of Cockcroft and Gault (5). Rapid screening tests for influenza virus (ZstatFlu; ZymeTx Inc., Oklahoma City, OK) were performed after enrollment for any subject who presented with influenza-like symptoms. Study data were recorded on case report forms and faxed to the VA Cooperative Studies Program Coordinating Center in Menlo Park, CA. Data were collected via DataFax (Clinical DataFax Systems Inc., Hamilton, Ontario) and placed into the study database for analysis.

Sample analysis. Oseltamivir phosphate, d_3-oseltamivir phosphate, oseltamivir carboxylate, and d_3-oseltamivir carboxylate were separated by using a newly developed high-performance liquid chromatography (HPLC) method and were detected by tandem mass spectrometry (MS-MS) with multiple-reaction monitoring (MRM). The HPLC-MS-MS analysis was performed with an Acquity ultraperformance liquid chromatography liquid handling system and a Quattro Premier XE triple quadrupole mass spectrometer (Waters Corp., Milford, MA) controlled by MassLynx (version 4.1) MS and chromatography manager software.

The separation was performed on an Acquity BEH C_{18} analytical column (2.1

by 100 mm; particle size, 1.7 μm) preceded by a Vanguard BEH C_{18} guard column (2.1 by 5 mm; particle size, 1.7 μm) (Waters Corp.) by using a mobile phase consisting of a 50:50 (vol/vol) mixture of 8.0 mM ammonium formate adjusted to pH 3.50 with formic acid and methanol at a flow rate of 0.200 ml/min. The Quattro premier mass spectrometer was used in the positive ion electrospray mode with a source temperature of 125°C, a desolvation temperature of 350°C, a desolvation gas flow of 700 liters/h, a cone gas flow of 9.0 liters/h, a capillary voltage of 3.20 kV, a cone voltage of 20 V, and a collision energy at 12.0 for the MRM experiments. Nitrogen was used as the nebulizer, auxiliary, and desolvation gas, while argon was used as the collision gas. The resolution was set at an 0.60-atomic-mass-unit width at half-height in both quadropole 1 and quadropole 3, and the analytes were detected by MRM with a 200-ms dwell time. MRM transitions were optimized by direct infusion of oseltamivir, d_3-oseltamivir, oseltamivir carboxylate, and d_3-oseltamivir carboxylate at a concentration of 100 ng/ml and a syringe pump flow rate of 10 μl/min. The optimal transitions were 313.14 $m/z \rightarrow$ 224.92 m/z for oseltamivir, 316.3 $m/z \rightarrow$ 228.3 m/z for d_3-oseltamivir, 285.2 $m/z \rightarrow$ 197.0 m/z for oseltamivir carboxylate, and 288.2 $m/z \rightarrow$ 200.2 m/z for d_3-oseltamivir carboxylate.

Oseltamivir phosphate and oseltamivir carboxylate as well as their respective internal standards, d_3-oseltamivir phosphate and d_3-oseltamivir carboxylate, respectively, were isolated from human plasma by a solid-phase extraction method with Oasis MCX 1-ml (30-mg) solid-phase extraction cartridges. Briefly, a plasma sample of 100 μl was mixed with the d_3-oseltamivir internal standard (0.50 μg/ml solution) and 20 μl of the d_3-oseltamivir carboxylate internal standard (10.0-μg/ml solution). The sample was vortexed for 5 s and centrifuged at 3,200 rpm and 4°C for 3 min. The samples were processed by using a RapidTrace solid-phase extraction module (Caliper Life Sciences, Hopkinton, MA). The cartridges were conditioned with 1.0 ml of Milli-Q H_2O (pH 3.0) and then 1.0 ml of methanol (pH 3.0). The samples were subsequently loaded onto the solid-phase extraction cartridges and rinsed with 1.0 ml of 2% formic acid and 1.0 ml of methanol, and finally, the sample was collected by elution with 1.0 ml of methanol with 5% NH_4OH. The eluent was collected in a clean test tube (12 by 75 mm) and evaporated to dryness with a Zymark TurboVap apparatus set at 40°C for 45 min. The sample was reconstituted with 70 μl of mobile phase, transferred into an HPLC vial, and placed in the autosampler tray at 4°C. An aliquot of 10.0 μl was injected into the HPLC system and eluted isocratically at 0.200 ml/min for 5 min by using a mobile phase consisting of (50:50, vol/vol) methanol and 8.0 mM $NH_4CH_3CO_2$ buffer at pH 3.50 with a column temperature of 26°C.

Probenecid concentrations were determined as follows. Briefly, the probenecid concentrations in human plasma samples (0.10 ml) were determined by a liquid chromatography-tandem MS procedure in a PE-Sciex API III system equipped with a Polaris C_{18} column (4.6 by 50 mm; particle size, 3 μm) and a mobile phase system consisting of acetonitrile-water-formic acid (55:45:0.06, vol/vol) and by mass spectrometric detection with the sample inlet heated with the nebulizer, positive ionization by atmospheric pressure chemical ionization, and mass scanning by MRM analysis. Sample preparation consisted of precipitation of 0.10 ml of plasma with acetonitrile containing the internal standard prior to separation by liquid chromatography-tandem MS. The standard curve range was 0.250 to 40.0 μg/ml, and the lower limit of quantitation was 0.250 μg/ml.

The precisions and accuracies of these assays were also evaluated. Calibration curves for oseltamivir phosphate and oseltamivir carboxylate were unweighted and were linear from 0.150 to 200 ng/ml and 5.0 to 10,000 ng/ml, respectively ($R^2 >$ 0.998). The limits of quantitation were 0.150 ng/ml and 5.0 ng/ml for oseltamivir phosphate and oseltamivir carboxylate, respectively; and the limits of detection were 0.10 ng/ml and 1.0 ng/ml, respectively. As a measure of accuracy, the errors were <15% and the inter- and intra-assay coefficients of variation were 3.31 to 10.48% and 2.63 to 9.03%, respectively, at three different drug concentrations. During the validation, the stability of the drug in plasma during repeated freezing and thawing of the samples was evaluated, and the overall level of recovery of all four analytes was >80%. Interday precision and accuracy measurements for the probenecid assay were determined by analyzing quality control samples made of human plasma spiked with known amounts of probenecid. Each of six sets (n = 2) of control samples at three different drug concentrations (30.0, 5.00, and 0.750 μg/ml) was evaluated (six standard curves were run). Precision, defined by the coefficient of variation, calculated as (standard deviation/mean) × 100, ranged from 5.92% to 7.59%. Accuracy, defined by the relative error, calculated as [(mean − nominal concentration)/nominal concentration] × 100, ranged from −3.67% to +5.60%.

Data analysis. Steady-state (day 15 to 16) oseltamivir and oseltamivir carboxylate concentrations were analyzed by standard noncompartmental methods with WinNonlin pharmacokinetic software (version 5.0; Pharsight Corporation, Mountain View, CA). The peak serum concentrations (C_{max}) of oseltamivir carboxylate and the time to reach C_{max} (T_{max}) were obtained directly by inspection of the concentration-time profiles. The trough concentration (C_{min}) was defined as the drug concentration in plasma immediately prior to the next scheduled dose (the concentration at 24 h [C_{24}] for group 1 and the concentration at 48 h [C_{48}] for groups 2 and 3). For groups 2 and 3, the steady-state predose oseltamivir carboxylate concentration (C_0) on dosing day 15 was imputed to the concentration at 48 h postdosing in place of the measured C_{48} (both concentrations represent trough values). This was done since C_0 was determined when probenecid was continued throughout the preceding dosing interval, whereas this was not the case for C_{48}. Since sampling was conducted under steady-state conditions, C_{min}s are expected to be consistent from dose to dose. On the basis of this principle, C_0 in place of the measured C_{48} was used to calculate the area under the concentration-versus-time curve over the course of the dosing interval (AUC_τ) for groups 2 and 3 and as the comparator C_{min} for groups 2 and 3, whereas C_{24} was used as the comparator C_{min} for group 1. To allow comparison of the values of AUC_τ between groups, the AUC from 0 to 24 h ($AUC_{0\text{-}24}$) for group 1 was doubled to yield the extent of oseltamivir carboxylate exposure over a 48-h period (i.e., the AUC from 0 to 48 h [$AUC_{0\text{-}48}$]) at steady state. The validity of this assumption rests on the fact that oseltamivir carboxylate was sampled under steady-state conditions for all groups. Since steady-state conditions are achieved after four to five $t_{1/2}$s and the $t_{1/2}$ of oseltamivir carboxylate is 7 to 10 h, steady-state conditions for this compound should have been reached within 2 days of the commencement of treatment. The apparent elimination rate constant (λ_Z) was estimated as the absolute value of the slope of a linear regression of the natural logarithm of the serum concentration-time profile. The $t_{1/2}$ was calculated as $\ln 2/\lambda_Z$. The AUC (AUC_τ is $AUC_{0\text{-}24}$ for group 1 and $AUC_{0\text{-}48}$ for groups 2 and 3) was determined by using the linear trapezoidal rule. All pharmacokinetic parameters are presented as geometric means with 90% confidence intervals or the arithmetic mean ± the standard deviation.

For the groups receiving probenecid (groups 2 and 3), average bioequivalence was assessed in accordance with standard methods by calculating the 90% confidence interval for the geometric mean ratios for the AUC at 48 h (AUC_{48}; between study groups 2 and 3) versus the AUC_{48} (the AUC at 24 h times 2) for the control group (group 1) (34). To establish bioequivalence, the calculated confidence interval must fall within the limit of 0.8 to 1.25.

Statistical analysis. The values of the pharmacokinetic parameters for oseltamivir carboxylate were compared between groups by analysis of variance (ANOVA) by the Tukey test. To compare the baseline characteristics of the subjects in the three groups, ANOVA was used for the continuous variables, such as age, weight, and laboratory values. For the categorical variables, a chi-square test or, when appropriate, Fisher's exact test was used. Significance was defined as a P value of <0.05 for all statistical comparisons.

RESULTS

Sixty-four subjects were enrolled at four clinical centers and were screened from April 2006 through March 2007. Fifty-three were randomized, and 48 were evaluable for pharmacokinetic analysis. All subjects who received at least one dose of the study medications were included in the safety population (n = 52). The characteristics of the subjects are listed in Table 1. There were 31 men and 22 women. The mean age for the entire cohort was 54 years, and 75% of the subjects were white. There were no significant differences in the characteristics of the subjects between study arms, including weight and creatinine clearance.

One subject was withdrawn for study drug compliance issues, one subject experienced a serious adverse event (see below), and another subject never received the study drug because the correct drug was not available at the site. Two additional subjects successfully completed the dosing phase without any adverse events but were determined to be not evaluable for the pharmacokinetic analysis due to the loss of their plasma samples as a result of freezer malfunction.

All three treatment regimens were well tolerated. The rate of adherence to the study medication ranged from 96 to 100% for all subjects and was not significantly different between the study arms. In general, there were no significant changes in

TABLE 1. Baseline subject demographics and physical characteristics by treatment group

Characteristic	Group 1	Group 2	Group 3	Total	*P* value
No. of subjects randomized	18	19	16	53	
Age					
Mean (SD)	55.6 (13.6)	54.1 (18.5)	52.4 (19.2)	54.1 (16.9)	0.8706
Median	56.5	62	55.5	56	
Range	28–75	23–79	22–76	22–79	
Sex (no. [%] of randomized subjects)					
Male	10 (56)	11 (58)	10 (63)	31 (58)	0.9173
Female	8 (44)	8 (42)	6 (38)	22 (42)	
Race (no. [%] of randomized subjects)					
White	13 (72)	12 (63)	15 (94)	40 (75)	0.6357
Black	2 (11)	3 (16)	0 (0)	5 (9)	
Hispanic	1 (6)	1 (5)	0 (0)	2 (4)	
Asian/Pacific Islander	1 (6)	2 (11)	0 (0)	3 (6)	
American Indian/Alaskan	0 (0)	0 (0)	0 (0)	0 (0)	
Multiple races	1 (6)	1 (5)	1 (6)	3 (6)	
Unknown	0 (0)	0 (0)	0 (0)	0 (0)	
Ht (in.)					
Mean (SD)	67.9 (3.4)	68 (3.7)	67.1 (4)	67.7 (3.6)	0.7459
Median	67.5	68	67.8	8	
Range	63–72	59–73	59–72	59–73	
Wt (lb)					
Mean (SD)	175.8 (41.8)	176.6 (29.4)	173 (36.1)	175.3 (35.3)	0.9553
Median	176.1	175.7	179.3	176.4	
Range	116.8–270.4	124.4–256.8	120.8–228.7	116.8–270.4	
Body mass index (kg/m^2)					
Mean (SD)	26.6 (4.9)	26.8 (3.7)	26.8 (3.8)	26.7 (4.1)	0.9825
Median	26.6	27	26.3	26.7	
Range	20.3–39.4	20.2–37.4	21.3–34.8	20.2–39.4	

blood chemistry, hematology indices, or calculated creatinine clearance. Serum uric acid levels were 42% lower at day 15 than at the baseline in groups 2 and 3 compared with the levels in group 1. Adverse events were generally mild and are listed in Table 2. A total of 45 events were recorded in 24 subjects after randomization. They were generally mild and were more frequent in group 2 than in groups 1 and 3. Of these, five events (four grade 1 gastrointestinal disorders) in two subjects were believed, in the opinion of the investigators, to be attributable to the study drug. One serious adverse event occurred in a 72-year-old female who developed grade 4 thrombocytopenia on day 15 of oseltamivir and probenecid QID dosing. Although she did not demonstrate any active bleeding, the study drug was discontinued and she was hospitalized overnight as a precaution. Her platelet count normalized within 1 week of study drug discontinuation.

The mean plasma concentration-time profiles for oseltamivir for group 1 and probenecid for groups 2 and 3 are shown in Fig. 1 and 2, respectively. The oseltamivir C_{max} and T_{max} values did not differ significantly between groups (data not shown), nor did the oseltamivir carboxylate C_{max}s (Table 3) or T_{max}s (data not shown) differ between groups, thereby suggesting that probenecid did not alter the absorption of oseltamivir or its systemic conversion to oseltamivir carboxylate. There was no significant difference in the values of the pharmacokinetic parameters for oseltamivir or oseltamivir carboxylate in group 1 compared to those in historical controls (arithmetic mean oseltamivir C_{max}s, 64 and 65 ng/ml, respectively; oseltamivir AUC_{0-24}s, 166 and 224 ng · h/ml, respectively; geometric mean oseltamivir carboxylate C_{max}s, 394 and 348 ng/ml, respectively; oseltamivir carboxylate AUC_{0-24}s, 5,873 and 5,438 ng · h/ml, respectively) (28; oseltamivir [Tamiflu] package insert; Roche, 2008).

The results of testing for average bioequivalence indicated that the 90% confidence intervals surrounding the geometric mean ratios of the oseltamivir carboxylate AUC_{48}s between groups 2 and 3 and group 1 were outside the bioequivalence criterion boundary (0.80 to 1.25) at 0.63 to 0.89 for group 2 versus that for group 1 and 0.57 to 0.90 for group 3 versus that for group 1. Significance testing did not show differences in the C_{max}s, $t_{1/2}$s, or AUC_τs for oseltamivir carboxylate between the study groups. Only the steady-state clearance of oseltamivir carboxylate was significantly reduced in groups 2 and 3 compared to that in group 1 ($P = 0.011$ and $P = 0.022$, respectively). The mean plasma concentration-time profiles of oseltamivir carboxylate for the three groups are shown in Fig. 3.

The average (arithmetic mean) C_{48}s for oseltamivir carboxylate are shown in Table 4. Of note, the mean C_{48} of oseltamivir carboxylate for group 1 was determined 48 h after the final oseltamivir dose. Although the mean C_{48} for group 2 (e.g., C_0) was approximately half of the mean C_{24} for group 1, these values were not significantly different ($P = 0.124$). Conversely,

TABLE 2. Adverse events after randomization and after randomization by attribution

Time to randomization and system organ class	Preferred term	Severity level	No. of events (no. of subjects)			
			Group 1	Group 2	Group 3	Total
After randomization						
Blood and lymphatic system disorders	Thrombocytopenia	4		1 (1)		
Gastrointestinal disorders	Constipation	1	1 (1)			
	Flatulence	1		1 (1)		
	Nausea	1	1 (1)	4 (3)		
	Vomiting	1		2 (1)	1 (1)	
General disorders and administration site conditions	Chest pain	1	1 (1)			
	Fever	1		1 (1)		
Infections and infestations	Upper respiratory infection	1	1 (1)			
Investigations	Creatinine level increase	1		1 (1)		
	Elevated liver enzyme levels	1		1 (1)	1 (1)	
	Urinalysis abnormal	1	1 (1)			
	Leukocyte level decrease	1	1 (1)	1 (1)	1 (1)	
Metabolism and nutrition disorders	Hyperkalemia	1			1 (1)	
Musculoskeletal and connective tissue disorders	Neck pain	2	1 (1)			
Renal and urinary disorders	Hematuria	1		3 (2)	2 (2)	
	Microscopic hematuria	1	1 (1)	4 (2)	5 (2)	
	Proteinuria	1	1 (1)			
Respiratory, thoracic, and mediastinal disorders	Runny nose	1		1 (1)		
Vascular disorders	Blood pressure fluctuation	1	1 (1)			
	Hypertension	1		1 (1)	1 (1)	
	Hypotension	2	1 (1)			
	Phlebitis	1		1 (1)		
After randomization subtotal			11 (7)	22 (10)	12 (7)	45 (24)
After randomization by attribution						
Possibly attributable						
Gastrointestinal disorders	Constipation	1	1 (1)			1 (1)
	Flatulence	1		1 (1)		1 (1)
	Nausea	1	1 (1)	2 (2)		3 (3)
	Vomiting	1			1 (1)	1 (1)
General disorders and administration site conditions	Chest pain	1	1 (1)			1 (1)
	Fever	1		1 (1)		1 (1)
Investigations	Creatinine level increase	1		1 (1)		1 (1)
	Leukocyte level decrease	1	1 (1)		1 (1)	2 (2)
Renal and urinary disorders	Hematuria	1			1 (1)	1 (1)
	Microscopic hematuria	1	1 (1)	2 (2)	1 (1)	4 (4)
Possibly attributable total			5	7	4	16 (10)
Positively attributable						
Blood and lymphatic system disorders	Thrombocytopenia	4		1 (1)		1 (1)
Gastrointestinal disorders	Nausea	1		2 (1)		2 (1)
	Vomiting	1		2 (1)		2 (1)
Positively attributable total				5 (2)		5 (2)
Total			11 (7)	22 (10)	12 (7)	45 (24)

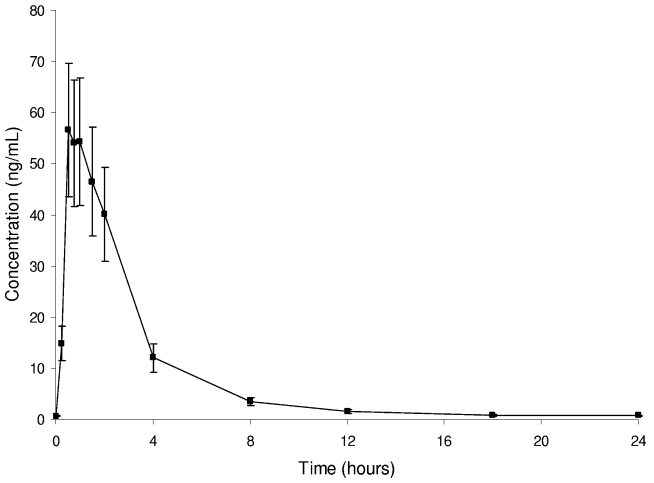

FIG. 1. Mean plasma oseltamivir concentrations (± standard error of the mean) versus time in 18 subjects receiving oseltamivir 75 mg daily for 2 weeks.

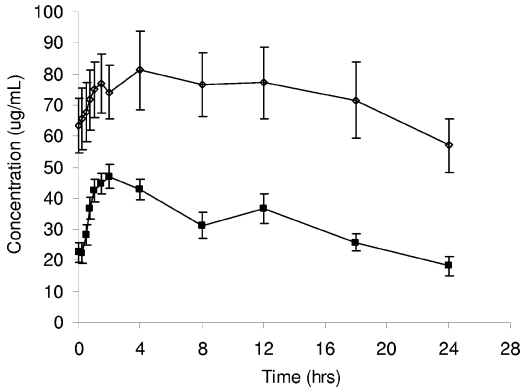

FIG. 2. Mean plasma probenecid concentrations (± standard error of the mean) versus time in subjects receiving probenecid 500 mg QID for 2 weeks (group 2; open diamonds) and 500 mg BID for 2 weeks (group 3; closed squares).

the mean C_{48} for group 3 was approximately 25% of the mean C_{24} for group 1, which was, in fact, significantly different ($P = 0.012$).

Consistent with the findings of other studies (1), the geometric mean AUC of oseltamivir carboxylate was higher in those subjects >65 years of age in all three groups but was significantly higher only in subjects in the arm receiving probenecid BID (geometric mean AUCs, 5,459 and 9,050 ng · h/ml, respectively; $P = 0.022$). In contrast, there were no significant differences in the pharmacokinetics of oseltamivir between males and females when the data were adjusted for total body weight (data not shown).

There was a linear relationship between the probenecid dose and the probenecid AUC. The probenecid AUC from time zero to infinity was 2.4 times higher in group 2 (2,177 μg · h/ml) than in group 3 (903 μg · h/ml), which is consistent with the findings obtained in previous dosing studies with a 500-mg dose (6, 29). Although other studies have demonstrated nonlinear kinetics, we did not study a dosing range sufficient to confirm this nonlinear relationship. Finally, there were no significant differences in the values of any of the oseltamivir or probenecid pharmacokinetic parameters when they were analyzed by clinical site.

DISCUSSION

Although the current prophylactic dose and duration of treatment with oseltamivir have been shown to effectively prevent influenza A virus infections in the majority of patients, alternative dosing strategies for prophylaxis have not been investigated. We have shown that oseltamivir given q48h in combination with probenecid given QID is safe and still maintains the plasma oseltamivir carboxylate concentrations within twofold of those for subjects who received the recommended dosage of oseltamivir given alone. This was not the case with the BID dosing of probenecid given in combination with oseltamivir.

The 50% inhibitory concentrations (IC_{50}s) (2) of oseltamivir carboxylate against influenza virus neuraminidases range from 0.3 to 22 nmol/liter (0.08 to 0.28 ng/ml). IC_{50}s against influenza virus strains in cell culture are somewhat higher and more variable and range from 0.6 to 155 nmol/liter (0.17 to 32.8 ng/ml) (26). Oseltamivir has also been shown to have activity against the H5N1 virus in vitro and in mouse challenge studies. The 50% effective concentrations against H5N1 strain replication in Madin-Darby canine kidney cells ranged from 7.5 to 12 μM, and the IC_{50}s for neuraminidase activity ranged from 7.0 to 15 nM (12, 18). The mean C_{min} of oseltamivir carboxylate after 48 h in the probenecid QID arm in our study was 42 ng/ml, which did not differ statistically from the C_{min} of oseltamivir carboxylate in the group that received oseltamivir but not probenecid. In addition, the AUC_{0-48}s and C_{max}s of the carboxylate metabolite were comparable between subjects receiving oseltamivir alone and those receiving oseltamivir in combination with probenecid QID ($P = 0.14$ and 0.65, respectively). These data suggest that oseltamivir carboxylate is likely to demonstrate significant antiviral activity in vivo when it is administered q48h in combination with probenecid given QID.

The data from our study indicate that when oseltamivir is combined with probenecid given QID, 75 mg of oseltamivir given q48h resulted in a C_{max}, C_{min}, and AUC_{48} of 394 ng/ml, 42 ng/ml, and 7.4 μg · h/ml, respectively. Prior studies performed with healthy volunteers indicated that multiple dosing of a 75-mg capsule given q24h resulted in a C_{max}, C_{min}, and AUC_{48} of 259 μg/liter or ng/ml, 39 μg/liter or ng/ml and 7.5 μg · hr/ml, respectively (oseltamivir [Tamiflu] package insert; Roche, 2008). This indicates that the concentrations are approximately 100-fold and 3- to 10-fold higher than those necessary for neuraminidase inhibition and influenza virus inhibition in cell culture, respectively. Although the C_{max} and AUC_{τ} of oseltamivir and its active metabolite in human subjects are useful parameters for confirmation of drug absorption and esterification, they are not necessarily good correlates of in vitro susceptibility or in vivo activity. In addition, although C_{min} would appear to be a relevant parameter for assessment of the relationship to the drug response in vivo, no data from human clinical trials have correlated oseltamivir carboxylate concentrations or any pharmacokinetic parameter with the virologic response or the clinical outcome (11; oseltamivir [Tamiflu] package insert; Roche, 2008).

TABLE 3. Summary of geometric mean pharmacokinetic parameters of oseltamivir carboxylate

Group	AUC (μg · h/ml)[a]	C_{max} (ng/ml)	CL_{ss}/F[b] (liters/h)	$t_{1/2}$ (h)
Group 1	9.75 (6.91–12.60)[c]	394 (287–392)	15.4 (12.0–18.8)	8.6 (7.00–10.2)
Group 2	7.4 (6.08–8.71)	394 (329–460)	10.3 (8.66–11.9)	9.5 (3.61–15.4)
Group 3	7.19 (6.41–7.98)	446 (384–507)	10.4 (8.5–12.4)	7.73 (6.26–9.21)
P[d] for group 2 vs group 1	0.14	0.65	0.015	0.51
P for group 3 vs group 1	0.12	0.78	0.023	0.97

[a] The AUC_{48}s for group 1 were calculated as the steady-state AUC_{24}s times 2; the AUC_{48}s for groups 2 and 3 were calculated by using C_0 as C_{48}, as described in the Materials and Methods.
[b] CL_{ss}/F, steady-state clearance.
[c] Values in parentheses are 90% confidence intervals.
[d] *P* values were determined by ANOVA and the Tukey post-hoc test.

In two prophylaxis studies conducted with healthy unvaccinated adults, the incidence of influenza virus infection was significantly reduced in those patients receiving 75 mg oseltamivir orally once daily for 42 days compared to the incidence in those receiving a placebo, although the current package insert suggests as few as 10 days may be sufficient (oseltamivir [Tamiflu] package insert; Roche, 2008). Several reports of uncontrolled trials suggest that 75 mg of oseltamivir given orally once daily for shorter prophylaxis courses appeared to prevent the appearance of influenza-like symptoms during an outbreak of non-avian influenza virus infection (25). Thus, depending on the influenza virus outbreak situation, the administration of a combination of oseltamivir and probenecid for 2 weeks could be an effective influenza prophylaxis regimen.

The side effect profiles reported from prior prophylaxis studies indicated that nausea and vomiting were more commonly found among subjects in the oseltamivir arm than among subjects in the placebo arm. The side effects among the subjects in our study were also generally mild. The frequency of gastrointestinal side effects was greater among subjects in the probenecid arms than among subjects in the control arm. However, between the two probenecid arms, the subjects in the BID arm had fewer gastrointestinal side effects than the subjects in the QID arm. In prior prophylaxis studies, there was also no difference in the incidence of side effects among populations of younger adults and populations of older adults (≥65 years of age) (oseltamivir [Tamiflu] package insert; Roche, 2008). We also did not find any greater incidence of side effects among the older subjects, even though subjects ≥65 years of age had active drug concentrations that were 25% higher than those in the younger adults. Finally, we did not see an increased incidence in renal dysfunction in the older subjects.

Although oseltamivir has been given to patients infected with the H5N1 variant, no controlled clinical trials with humans infected with or requiring prophylaxis for the H5N1 variant have been performed. Oseltamivir treatment of H5N1 influenza pneumonia in a pediatric patient has been published. Oseltamivir was given late in the course of illness, and the child subsequently died (8). Several people were given a prophylactic course of oseltamivir after avian influenza virus (H7N7, H7N3) outbreaks in The Netherlands and British Columbia, Canada, which appeared to be effective in preventing additional human cases (16, 32). Another pharmacokinetic clinical trial is under way (Clinicaltrials.gov identifier NCT00439530) and is evaluating additional strategies of combining oseltamivir and probenecid in Thai subjects.

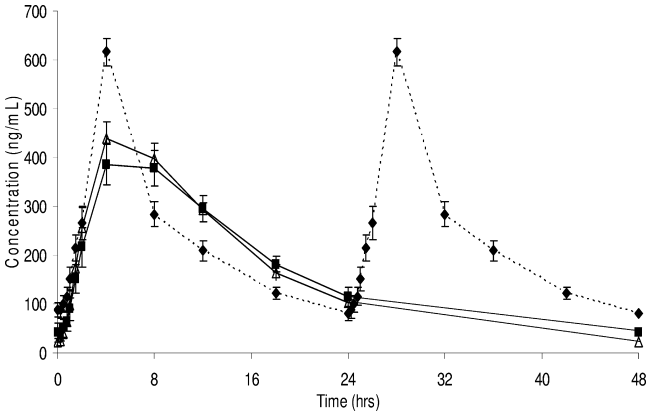

FIG. 3. Mean plasma oseltamivir carboxylate concentrations (± standard error of the mean) versus time in three groups of healthy human subjects after 2 weeks of oseltamivir administration. Group 1 (solid diamonds, dashed line) received oseltamivir 75 mg q24h; group 2 (solid squares, solid line) received oseltamivir 75 mg q48h plus probenecid 500 mg QID; and group 3 (open triangles, solid line) received oseltamivir 75 mg q48h plus probenecid 500 mg BID. Error bars indicate standard errors of the means.

TABLE 4. Predose and trough oseltamivir carboxylate concentration comparisons

Group (conc measured)	Mean ± SD concn (ng/ml)	No. of subjects with concn <300 ng/ml/ total no. of subjects (%)	*P* value[a] vs C_{24} for group 1	*P* value vs C_{48} for group 1
Group 1 (C_{48}, 24-h dose skipped)	14 ± 16	6/18 (33)		
Group 1 (C_{24})	81 ± 54	0/18 (0)		
Group 2 (C_0, i.e., C_{48}; predose)	42 ± 76	1/16 (6)	0.124	0.194
Group 3 (C_0, i.e., C_{48}; predose)	23 ± 26	2/16 (13)[b]	0.012	0.841

[a] *P* values were determined by ANOVA.
[b] Two subjects had concentrations between 300 and 400 ng/ml.

Recent reports indicate that the esterases in plasma could result in increased oseltamivir carboxylate concentrations in human plasma samples not properly processed in sufficient time or not collected in the proper blood collection tube (20, 21). All of our samples were processed and frozen within 30 min of collection. Although the oseltamivir carboxylate concentrations were lower in the two probenecid arms, given our sample processing technique, it is unlikely that a loss of oseltamivir in these samples because of endogenous esterase activity would be a factor to explain the lower oseltamivir carboxylate concentrations.

Solely on the basis of the data from the current investigation, probenecid in combination with oseltamivir cannot be considered for use by all patients requiring oseltamivir prophylaxis, given the number of medications and medical conditions that could be affected by probenecid. Prior studies have indicated that there is no significant interaction of probenecid with hepatic P450 isoenzymes. However, numerous medications are renally excreted and could be affected by probenecid coadministration. Although both QID and BID dosing of probenecid significantly reduced the clearance of oseltamivir by approximately 30%, only QID dosing was able to maintain the oseltamivir carboxylate C_{min} within twofold of the C_{min} for conventional q24h oseltamivir dosing. The current study was limited in that it was not designed to provide a thorough characterization of probenecid administration (i.e., dose, plasma exposure, and time course) on oseltamivir disposition. Pharmacokinetic modeling studies assessing the influence of probenecid administration as well as additional covariates (age, gender, renal function, etc.) on the pharmacokinetics of oseltamivir are required to determine the optimal means of coadministering these two drugs and whether there is a role for dosing oseltamivir every other day in combination with probenecid for prophylaxis for influenza virus.

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