Pharmacokinetic-Pharmacodynamic Determinants of Oseltamivir Efficacy Using Data from Phase 2 Inoculation Studies

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Given the limited understanding about pharmacokinetic-pharmacodynamic (PK-PD) determinants of oseltamivir efficacy, data from two phase 2 influenza virus inoculation studies were evaluated. Healthy volunteers in studies 1 and 2 were experimentally infected with influenza A/Texas (the concentration of neuraminidase inhibitor which reduced neuraminidase activity by 50% [IC50] = 0.18 nM) or B/Yamagata (IC50 = 16.76 nM), respectively. In study 1, 80 subjects received 20, 100, or 200 mg of oral oseltamivir twice daily (BID), 200 mg oseltamivir once daily, or placebo for 5 days. In study 2, 60 subjects received 75 or 150 mg of oral oseltamivir BID or placebo for 5 days. Oseltamivir carboxylate (OC) (active metabolite) PK was evaluated using individual PK data and a population PK model to derive individual values for area under the concentration-time curve from 0 to 24 h (AUC0–24), minimum concentration of OC in plasma (Cmin), and maximum concentration of OC in plasma (Cmax). Exposure-response relationships were evaluated for continuous (area under composite symptom score curve [AUCSC], area under the viral titer curve, and peak viral titer) and time-to-event (acceleration of composite symptom scores and cessation of viral shedding) efficacy endpoints. Univariable analyses suggested the existence of intuitive and highly statistically significant relationships between OC AUC0–24 evaluated as a 3-group variable and AUCSC, time to alleviation of composite symptom scores, and time to cessation of viral shedding. The upper OC AUC0–24 threshold (~14,000 ng · h/ml) was similar among these endpoints. Multivariable analyses failed to demonstrate the influence of study/strain on efficacy endpoints. These results provide the first demonstration of exposure-response relationships for efficacy for oseltamivir against influenza and suggest that OC exposures beyond those achieved with the approved oseltamivir dosing regimen will provide enhanced efficacy. The clinical applicability of these observations requires further investigation.

Oseltamivir is an orally available prodrug of the influenza virus neuraminidase inhibitor (NAI), oseltamivir carboxylate (OC). While oseltamivir has been studied extensively, its efficacy and safety profile is known, and it has been licensed for more than a decade for the treatment and prophylaxis of influenza in persons aged ≥1 year (1, 2), fundamental information describing relationships between OC exposure and clinical and virologic response in humans is lacking. Such information is important, as it forms part of the scientific basis for optimizing treatment regimens for influenza, especially for viruses possessing greater pathogenicity than regular seasonal strains.

Indirect information from preclinical systems provides some important insights about pharmacokinetic-pharmacodynamic (PK-PD) relationships for OC and other NAIs, including zanamivir and peramivir. McSharry et al. and Brown et al. utilized an in vitro pharmacodynamic hollow-fiber infection model (HFIM) system to explore PK-PD determinants of oral oseltamivir (3) and intravenous (i.v.) zanamivir (4, 5) antiviral activity. In dose fractionization study designs for OC, the treatment schedule did not appear to alter suppression of viral replication, thus indicating that the PK-PD index associated with efficacy was the AUC0–24/EC50 ratio, the ratio of the area under the concentration-time curve from 0 to 24 h (AUC0–24) to the drug concentration that reduces the number of plaque forming units (PFU) by 50% (EC50). In contrast, it was demonstrated that time above EC50 was the PK-PD index predictive of efficacy for the i.v. administration of zanamivir. The differences in these results were attributed to differences in the half-lives of the respective NAIs. Interestingly, when the i.v. zanamivir half-life was increased from 2.5 to 8 h (i.e., the same as the OC half-life), the PK-PD-linked index was the AUC0–24/EC50 ratio. The authors speculated that for oseltamivir, it may be possible to effectively treat influenza with a once-a-day schedule. Interestingly, the authors also noted an exposure-response relationship for OC that was less steep than that for other non-NAI antiviral agents, suggesting there may be opportunity to identify higher doses that may provide additional virologic benefit.

In vivo investigations in mice and ferrets have also provided some supportive information on exposure-response relationships for OC, with much of the published information reported from investigations in highly pathogenic virus subtypes. In ferrets lethally challenged with highly pathogenic influenza A/Vietnam/1203/04 (H5N1) given oseltamivir treatment initiated 24 h postinfection, dosing regimens providing similar exposures to those achieved by giving 75 mg twice daily (BID) (approved dosing regimen) in humans were insufficient to prevent death; doses 2.5-fold higher were necessary to prevent death in ferrets in this model (6).
Similar observations were noted in mice challenged with differing H5N1 clades, experiments for which oseltamivir treatment was initiated 4 h prior to inoculation (7). Logistical constraints make it difficult to obtain PK for PK-PD examinations directly from animals infected with such highly virulent viruses. Thus, a limitation for such studies is that the inferences are based primarily on dose-response data and PK is inferred. Recently, a PK-PD evaluation was performed in ferrets inoculated for influenza B/Yamagata/1988 in which both OC PK and PD were determined. In contrast to more virulent strains, only mild disease was induced following inoculation, thereby limiting the ability to detect PK-PD relationships. Despite this limitation, the authors noted a PK-PD association between increasing OC AUC and positive impact on the weight of ferrets in this study (8).

In summary, the available preclinical findings from HFIM and in vivo infection models suggest the existence of exposure-response relationships for efficacy for OC. These data also suggest that AUC is the more important exposure measure associated with OC efficacy. However, there is no clear evidence that the maximal effect $(E_{\text{max}})$ has been attained for all influenza viruses at OC exposures comparable to those achieved with the labeled oseltamivir dosing regimen of 75 mg BID.

In an effort to further characterize the exposure-response relationships between OC exposure and virologic and clinical efficacy endpoints, analyses were undertaken using data from two well-controlled phase 2 influenza virus inoculation studies performed in healthy volunteers (9, 10).

(Results of this investigation were presented at the XIV International Symposium on Respiratory Viral Infections, 23 to 26 March 2012, Istanbul, Turkey.)

**MATERIALS AND METHODS**

**Study population, drug dosage, and administration.** The study population included data from two phase 2 studies, study PV15616 (study 1) and study NP15717 (study 2), evaluating the safety, tolerability, and antiviral activity of various doses of oseltamivir in healthy adults experimentally inoculated with either influenza virus A/Texas/36/91 (H1N1) or influenza virus B/Yamagata/16/88, respectively (9, 10). Study 1 was a single-center, multiple-dose, double-blind, randomized, placebo-controlled study which evaluated four oral oseltamivir dosing regimens, 20 mg BID for 5 days, 100 mg BID for 5 days, 200 mg BID for 5 days, 200 mg once daily (QD), and matching placebo administered BID for 5 days (9). Study 2 was a single-center, multiple-dose, double-blind, randomized, placebo-controlled study evaluating two oral dosing regimens of oseltamivir, 75 mg BID and 150 mg BID, and placebo given orally BID for 5 days (10).

The inclusion criteria for both studies were similar. Healthy adult subjects enrolled in these studies were ≥18 years of age, had an influenza virus antibody level of ≤1:8 (study 1) or ≤1:10 (study 2), had no significant health abnormalities as determined by evaluation of medical history, general physical exam, vital signs, laboratory tests, and electrocardiogram, were nonsmokers or consumed an average of less than 10 cigarettes per day (study 1), and were able to give informed consent.

Healthy subjects were excluded if they met any of the following criteria: had hepatitis B infection; were transplant recipients; were taking steroids or receiving immunosuppressant therapy; had a known HIV infection; had a hypersensitivity to oseltamivir or structurally similar compounds; had a known allergy to components of the virus suspension; were asthmatic and were receiving chronic therapy for asthma; had experienced a previous episode of acute respiratory tract infection, otitis, bronchitis, or sinusitis or had received antibiotics for any of these conditions within 2 weeks prior to the start of the study; had pyrexia within 3 days of study start; had a clinically relevant history of alcohol or drug abuse; or had been given an influenza vaccine less than 6 months prior to study start (9, 10).

Concomitant medications that might interfere with oseltamivir metabolism, gut motility, or renal excretion were excluded. Healthy subjects were allowed to receive paracetamol for the relief of fever and discomfort, and continued use of the oral contraceptive pill was permitted during the study. Other medications required for disease symptoms or other medical conditions arising during the study were permitted at the discretion of the investigators.

The two studies were carried out in accordance with the principles of the Declaration of Helsinki and applicable local laws. Full ethical committee approval was obtained.

**Schedule of assessments.** In study 1, healthy subjects underwent screening within 4 weeks prior to the first dose during which a complete medical history, physical examination, vital signs, safety laboratory samples (hematology, biochemistry, and hepatitis B and HIV antibodies), urine drug screen, and urinalysis were conducted. A sample size of 80 subjects was chosen, and subjects were randomized to one of five treatment groups. Subjects were admitted to the isolation unit on day 1 and remained in the unit until discharge on the morning of day 9 (9). Vital signs, blood samples for safety laboratory samples and hemagglutination inhibition antibody titers, urine samples for urinalysis, nasal wash samples for virus culture and titer, and nasal discharge weights were taken prior to inoculation. A physical exam was performed prior to inoculation, and symptom assessment scores (self-rated by the subject) were assessed prior to inoculation. Inoculation with the human influenza virus A/Texas/36/91 (H1N1) was performed on the evening of day 1 via nasal drops containing a median tissue culture infective dose $(\text{TCID}_50)$ of virus of $10^7$, following which repeat PD assessments (as described below), vital signs, and temperature were recorded (9).

Treatment with study drug was initiated on the evening of day 2, 28 h after inoculation. On days 2 to 8, the following assessments were performed: adverse event history; modified physical examination if indicated by the adverse event history or medical evaluation; twice daily vital signs measurements; temperature recordings 4 times daily; twice daily symptom score self-assessment (nasal stuffiness, ear ache, runny nose, sore throat, cough, breathing difficulty, myalgia, fatigue, headache, feeling feverish, hoarseness, sneezing, chest discomfort, and overall discomfort); nasal wash samples for viral culture and viral titer taken twice daily on days 2 and 3 and once daily thereafter. On day 9, after completion of the assessments as taken on days 2 to 8 in addition to blood for safety samples, subjects were allowed to leave the isolation unit at the discretion of the supervising physician and if the symptoms of influenza had resolved. If symptoms persisted, subjects remained in the isolation unit and were monitored until the resolution of all symptoms. Three to 4 weeks after virus inoculation, subjects returned to the clinic for a final assessment which included an adverse event history and blood sampling to determine virus antibody titer (9).

The study schedule for study 2 was similar to that of study 1 with the following exceptions: subjects were screened within 2 weeks prior to the start of the study, and a sample size of 60 subjects with baseline antibody titers of <1:10 was chosen with 20 subjects randomized to each of the three treatment groups; subjects were admitted to the isolation unit 24 h (day $-1$) prior to inoculation with influenza B/Yamagata/16/88 virus and remained in the isolation unit until discharge on day 8; subjects underwent twice daily nasal washes for virus culture on days 1 to 3 and then once daily from days 4 to 8; and blood samples for the assessment of laboratory endpoints for safety were collected on days 3 and 5 (10).

NA inhibition assay was performed with viruses standardized to equivalent NA activity and incubated with NAIs at concentrations of 0.00005 to 100 μM with 2’-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid as a substrate (final concentration of 100 μM). The $I_{50}$ was determined by plotting the dose-response curve of inhibition of NA activity at 60 min as a function of the compound concentration. Each experiment was performed in triplicate, and the final mean ± standard
deviation (SD) IC₅₀ was 16.76 ± 4.10 nM for influenza B/Yamagata and 0.18 ± 0.11 nM for influenza A/Texas.

**Efficacy endpoints.** PK-PD analyses described below involved the evaluation of five efficacy endpoints. These endpoints included three continuous and two time-to-event variables. The continuous efficacy endpoints included composite symptom score AUC, viral titer AUC, and peak viral titer. The composite symptom score was calculated using seven individual symptom scores, including feeling feverish, headache, muscle ache, sore throat, cough, overall discomfort, and nasal symptoms (defined as the maximum of “nasal stuffiness” or “runny nose”) (10). During the first 9 days of the study, each subject self-rank the severity of each symptom twice daily using a scale for which 0 represents the absence of symptoms and 3 represents the most severe symptoms. The seven scores were added to form the composite symptom score. The AUC of this composite symptom score-versus-time curve over 9 days was likewise calculated using the linear trapezoidal rule. The AUC of the viral titer-versus-time curve over 9 days was likewise calculated using the linear trapezoidal rule. Peak viral titer was defined as the maximum viral titer value for each subject observed during the study period.

The time-to-event efficacy endpoints included time to alleviation of the composite symptom score and time to cessation of viral shedding. The time to alleviation of the composite symptom score was calculated as the time at which any of the seven individual symptom scores, as described above, was rated as >1 (time = 0) until the time at which all applicable individual symptom scores were ≤1. The time to cessation of viral shedding was calculated using the viral culture data acquired using nasal lavage. The time to cessation of viral shedding was defined as the time from the first positive viral culture result (time = 0) until the time of the first negative viral culture result.

**Pharmacokinetic sampling.** In study 1, plasma samples for PK assessment of oseltamivir and OC were taken prior to the morning dose of medication on days 3, 4, and 7. In study 2, samples for PK assessment were taken each morning just prior to drug administration on days 2 to 4 and complete 12-hour plasma drug PK profiles were obtained following the first dose on days 1 and 5. Additional details describing the plasma drug sample assay are provided elsewhere (11).

**Determination of plasma drug exposures.** The steady-state AUC₀₋₂₄ and the maximum and minimum plasma OC concentrations (Cₘₐₓ and Cₘᵟᵟᵟ, respectively) represented the exposure measures evaluated for the PK-PD analyses described herein. In the accompanying article by Kamal et al. (11), a population PK model for oseltamivir and OC, which was based on data from 13 clinical studies, including the two studies described herein, was used to obtain post hoc PK parameter estimates for the subjects in the current analysis. Using these post hoc PK parameter estimates, exposure measures were computed for each subject (11). In brief, the model simultaneously described the plasma PK data for both oseltamivir and OC using two compartments for oseltamivir with first-order absorption and direct conversion of oseltamivir to OC and one compartment for OC with first-order elimination. A covariate analysis demonstrated that weight and creatinine clearance, and to a lesser degree age, were statistically significant predictors of the PK of oseltamivir and OC. As evidenced by the agreement between both the population mean predicted (r² = 0.741) and individual predicted (r² = 0.969) and observed plasma OC concentrations, the model fit the data well (see Figure S1 in the supplemental material).

**Pharmacokinetic-pharmacodynamic analyses.** Univariable and multivariable PK-PD analyses were conducted as described below using R 2.11.1 (12), and data from all evaluable subjects in the two studies, including those who received placebo. Evaluable subjects were those for whom OC AUC₀₋₂₄ values could be computed and for whom adequate data for at least one efficacy endpoint were available. Subjects who received placebo and for whom efficacy data were available were also considered; an OC AUC₀₋₂₄ of zero was assumed for these subjects.

**Univariable analyses.** Univariable relationships for each continuous efficacy endpoint were evaluated using the F-test from linear regression or analysis of variance. Univariable relationships for each time-to-event efficacy endpoint were examined using log rank tests for categorical independent variables and the likelihood ratio test from Cox proportional hazard regression for continuous independent variables.

The independent variables evaluated in these analyses included the OC exposure variables, AUC₀₋₂₄, Cₘₐₓ, Cₘᵟᵟᵟ, and IC₅₀. Each continuous OC exposure variable was evaluated in its original form and as 2- and 3-group categorical variables to account for potential nonlinearity and/or nonmonotonicity. The categorical forms of these independent variables were constructed using thresholds that were optimally determined for the given efficacy endpoint. Two-group independent variables were constructed by using the resulting split of a regression tree for a continuous efficacy endpoint and by using a cutoff maximizing the log rank test derived from a univariable Cox proportional hazard regression model for a given time-to-event efficacy endpoint. Three-group independent variables were constructed by determining a pair of cutoff values that minimized the likelihood ratio P value using linear regression for a continuous efficacy endpoint. For a time-to-event efficacy endpoint, minimization of the log rank P value derived from Cox proportional hazard regression was used to determine the pair of cutoff values to define the 3-group independent variable. For both 2- and 3-group independent variables, a minimum subgroup size of 10 subjects was imposed to construct such categorical variables. OC exposure measures were also evaluated as an empirically divided categorical variable; each measure was divided into quartiles. IC₅₀, which included two values, 0.18 and 16.76 nM, was evaluated as a categorical variable.

**Multivariable analyses.** Multivariable analyses were carried out for each efficacy endpoint, with consideration of separate models that included each of the above-described exposure measures and IC₅₀. Each continuous efficacy endpoint was analyzed using linear regression, while each time-to-event efficacy endpoint was analyzed using Cox proportional hazard regression. Multivariable models considering the interaction between individual forms of OC exposure and IC₅₀ were also assessed. The statistical significance of the model parameters was tested using Wald P values for single parameters (e.g., 2-group and continuous forms of an independent variable) and likelihood ratio P values for multiple parameters (e.g., 3-group and quartile forms of an independent variable). Model discrimination was accomplished using the corrected Akaike’s information criterion (AICc) (13), an assessment that balances improvement in goodness of fit with model complexity (e.g., degrees of freedom, number of fitted parameters). If the AICc value was closely similar among more than one model, the final multivariable model was chosen based on clinical judgment, including the biological plausibility of the nature of the relationships for OC exposure or IC₅₀ retained in the model.

**RESULTS**

**Subject population.** A total of 115 subjects were evaluated for inclusion in these analyses. In study 1, 69 (45 female and 24 male) subjects received either active treatment (n = 56) or placebo (n = 13) and were inoculated with influenza A/Texas/36/91 (H1N1) virus. For study 2, 46 (14 female and 32 male) subjects received either active treatment (n = 30) or placebo (n = 16) and were inoculated with influenza B/Yamagata/16/88 virus. All 86 subjects who received study drug had PK data available. Summary statistics of baseline characteristics for all evaluable subjects, stratified by study, are presented in Table 1.

**Summary of exposure measures.** Figure 1 shows the comparison of distribution of the OC AUC₀₋₂₄ values for all evaluable subjects by study as represented by box plots. While the median AUC₀₋₂₄ values were closely similar, there was a wider range of
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TABLE 1 Summary statistics of baseline demographic characteristics for all evaluable subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Study 1</th>
<th>Study 2</th>
<th>Both studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>22.3 (20.3)</td>
<td>25.0 (29.3)</td>
<td>23.4 (25.4)</td>
</tr>
<tr>
<td>Ht (cm)</td>
<td>170 (5.72)</td>
<td>176 (44.4)</td>
<td>172 (5.45)</td>
</tr>
<tr>
<td>Wt (kg)</td>
<td>68.6 (20.5)</td>
<td>73.7 (13.4)</td>
<td>70.6 (18.0)</td>
</tr>
<tr>
<td>Creatinine clearance (ml/min/1.73 m²)</td>
<td>108 (23.8)</td>
<td>124 (17.8)</td>
<td>114 (22.2)</td>
</tr>
</tbody>
</table>

Sex
- Male: 35.0 (24/69) 70.0 (32/46) 49.0 (56/115)
- Female: 65.0 (45/69) 30.0 (14/46) 51.0 (59/115)

Race
- Black: 11.6 (8/69) 0 (0/46) 6.96 (8/115)
- White: 78.3 (54/69) 93.5 (43/46) 84.4 (97/115)
- Other: 10.4 (7/69) 6.50 (3/46) 8.70 (10/115)

Total daily dose (mg)
- 40: 21.7 (15/69) 0 (0/46) 13.0 (15/115)
- 150: 0 (0/69) 32.6 (15/46) 13.0 (15/115)
- 200: 39.1 (27/69) 0 (0/46) 23.5 (27/115)
- 300: 0 (0/69) 32.6 (15/46) 13.0 (15/115)
- 400: 20.3 (14/69) 0 (0/46) 12.2 (14/115)
- None (placebo): 18.8 (13/69) 34.8 (16/46) 25.2 (29/115)

Treatment regimen (mg)
- 20 BID: 21.7 (15/69) 0 (0/46) 13.0 (15/115)
- 75 BID: 0 (0/69) 32.6 (15/46) 13.0 (15/115)
- 100 BID: 20.3 (14/69) 0 (0/46) 12.2 (14/115)
- 150 BID: 0 (0/69) 32.6 (15/46) 13.0 (15/115)
- 200 QD*: 18.8 (13/69) 0 (0/46) 11.3 (13/115)
- 200 BID: 20.3 (14/69) 0 (0/46) 12.2 (14/115)
- None (placebo): 18.8 (13/69) 34.8 (16/46) 25.2 (29/115)

*QD, once daily.

TABLE 2 Comparisons of OC AUC₀⁻²⁴ values for all evaluable subjects administered active treatment stratified by study. The OC AUC₀⁻²⁴ values are shown in nanograms · hour/milliliter. The box shows the 25th to 75th percentile values and the line in the box shows the median value, while the whiskers extend from the minimum and maximum values.

![FIG 1 Comparison of OC AUC₀⁻²⁴ values for all evaluable subjects administered active treatment stratified by study. The OC AUC₀⁻²⁴ values are shown in nanograms · hour/milliliter. The box shows the 25th to 75th percentile values and the line in the box shows the median value, while the whiskers extend from the minimum and maximum values.](http://aac.asm.org/)

- Exposures for subjects in study 1 than for those in study 2 as would be expected given the dose ranges for each study. When examining the Spearman rank correlation coefficient among OC AUC₀⁻²⁴, Cmax, and Cmin, high correlations among all three exposure measures were evident (>0.96). Given these findings and the fact that the PK-PD index reported to be the most associated with the efficacy of NAIs in preclinical models was the AUC₀⁻²⁴/EC₅₀ ratio (3, 4), univariable and multivariable analyses described herein were carried out using OC AUC₀⁻²⁴.

Pharmacokinetic-pharmacodynamic analyses. (i) Univariable analyses. A summary of the P values and directional assessments for the univariable relationships between the efficacy endpoints and OC AUC₀⁻²⁴ evaluated as continuous and 2-, 3-, and quartile categorical variables or IC₅₀ (categorical variable), is shown in Table 2. Scatterplots showing the relationship between each continuous efficacy endpoint and OC AUC₀⁻²⁴ evaluated as a continuous variable, with the OC AUC₀⁻²⁴ ranges encompassing the 2- and 3-group variables indicated by a triangle symbol and dashed vertical lines, respectively, are presented in Fig. S2 in the supplemental material.

Significant relationships with at least one form of OC AUC₀⁻²⁴ were evident for all five endpoints. For four of the endpoints, composite symptom score AUC, viral titer AUC, time to alleviation of composite symptom score, and time to cessation of viral shedding, the relationships with OC AUC₀⁻²⁴ evaluated as a 2- and/or 3-group variable incorporated comparisons with the lowest OC AUC₀⁻²⁴ group, which was mostly or entirely comprised of subjects who received placebo. However, in the case of composite symptom score AUC and time to cessation of viral shedding and alleviation of composite symptom score, statistically significant 3-group categorical assessments demonstrated increased response between the middle and higher OC AUC₀⁻²⁴ groups. Biologically plausible univariable relationships between viral titer AUC or peak viral titer and OC AUC₀⁻²⁴ were not apparent.

Univariable relationships for IC₅₀ are also presented in Table 2. However, given that OC AUC₀⁻²⁴ were not balanced across studies (as shown in Fig. 1), none of the endpoints demonstrated increased response with IC₅₀. Thus, the evaluation of the impact of OC AUC₀⁻²⁴ and IC₅₀ were assessed as part of the multivariable analyses.

Parameter or hazard ratio estimates for univariable models describing the relationship between composite symptom score AUC, time to alleviation of composite symptom score, or time to cessation of viral shedding and OC AUC₀⁻²⁴ evaluated as a 3-group variable are provided in Table 3. As shown in Table 4, the influence of the middle and higher OC AUC₀⁻²⁴ groups relative to the lowest OC AUC₀⁻²⁴ group on the efficacy endpoints is demonstrated by comparing the time (in days) to 25, 50, and 75% of the population achieving time to alleviation of composite symptom score or time to cessation of viral shedding or the mean value for composite symptom score AUC among OC AUC₀⁻²⁴ groups. The above-described univariable relationships are also shown graphically by stratified Kaplan-Meier curves for time to alleviation of composite symptom score and time to cessation of viral shedding and by box plots for composite symptom score AUC by OC AUC₀⁻²⁴ group in Fig. 2.

Highly statistically significant relationships between each of the three efficacy endpoints and OC AUC₀⁻²⁴ evaluated as a 3-group variable were apparent (P < 0.007). Not surprisingly, the OC AUC₀⁻²⁴ thresholds for composite symptom score AUC and time to alleviation of composite symptom score when OC
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AUC<sub>0–24</sub> was evaluated as a 3-group categorical variable were similar (≤1,495, >1,495 to ≤14,497, >14,497 and ≤1,568, >1,568 to ≤13,638, >13,638 ng · h/ml, respectively). OC AUC<sub>0–24</sub> thresholds for time to cessation of viral shedding were ≥0 to ≤14,180, and >14,180 ng · h/ml. The highest group thresholds for the OC AUC<sub>0–24</sub> were similar for time to cessation of viral shedding, composite symptom score AUC, and time to alleviation of composite symptom score (>14,180, >13,638, and >14,497 ng · h/ml, respectively).

The influence of higher OC AUC<sub>0–24</sub> on lower symptom score AUC was evident as assessed by the mean values for composite symptom score AUC, which were 14.6, 9.1, and 6.7 for OC AUC<sub>0–24</sub> groups with threshold values of ≤1,495, >1,495 to ≤14,497, and >14,497 ng · h/ml, respectively. The influence of higher AUC<sub>0–24</sub> on earlier time-to-event variables was evident as assessed by the lengths of time for 50% of the population to achieve alleviation of their composite symptoms, which were 0.75, 1.5, and 3.5 days for OC AUC<sub>0–24</sub> groups with threshold values of >13,368, >1,568 to ≤13,368, and ≤1,568 ng · h/ml, respectively. The influence of higher OC AUC<sub>0–24</sub> on earlier time to cessation of viral shedding was evident as assessed by the lengths of time for 50% of the population to achieve this endpoint, which were 2, 2.5, and 4.5 days for OC AUC<sub>0–24</sub> groups with threshold values of >14,180, >14,180 to ≤14,180, and 0 ng · h/ml, respectively.

(ii) Multivariable analyses. A summary of the P values and directional assessments for the relationships between the efficacy endpoints and OC AUC<sub>0–24</sub>, adjusted for IC<sub>50</sub>, based on multivariable linear regression or Cox regression models, is provided in Table 5. For each efficacy endpoint, AIC<sub>c</sub> values and the nature of each exposure-response relationship for OC AUC<sub>0–24</sub> evaluated in a given form were assessed to discriminate among candidate models. For models for which AIC<sub>c</sub> values were statistically indistin-

### Table 2: Summary of P values and directional assessments for univariable relationships between efficacy endpoints and OC AUC<sub>0–24</sub> or IC<sub>50</sub>

<table>
<thead>
<tr>
<th>Efficacy endpoint</th>
<th>Directionality and P value for efficacy endpoint&lt;sup&gt;a&lt;/sup&gt;</th>
<th>OC AUC&lt;sub&gt;0–24&lt;/sub&gt;</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Quartile</th>
<th>Continuous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite symptom score AUC</td>
<td>/, &lt;0.001</td>
<td>/, &lt;0.001</td>
<td>/, 0.036</td>
<td>/, 0.010</td>
<td>, 0.06</td>
</tr>
<tr>
<td>Viral titer AUC</td>
<td>/, 0.004</td>
<td>/, 0.010</td>
<td>N, 0.042</td>
<td>/, 0.06</td>
<td>, 0.035</td>
</tr>
<tr>
<td>Peak viral titer</td>
<td>/, 0.049</td>
<td>, 0.004</td>
<td>0.26</td>
<td>0.55</td>
<td>/, 0.011</td>
</tr>
<tr>
<td>Time to alleviation of composite symptom score</td>
<td>/, &lt;0.001</td>
<td>/, &lt;0.001</td>
<td>/, 0.006</td>
<td>/, &lt;0.001</td>
<td>, 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Time to cessation of viral shedding</td>
<td>/, 0.012</td>
<td>/, 0.007</td>
<td>/, 0.09</td>
<td>/, 0.037</td>
<td>, 0.026&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Summary of P values and directional assessments for univariable relationships between efficacy endpoints and OC AUC<sub>0–24</sub> evaluated as a continuous, 2-group, 3-group, and quartile categorical variables or IC<sub>50</sub>

<sup>b</sup> As described in Materials and Methods for the univariable analyses, P values for univariable relationships for continuous efficacy endpoints were based on the F-test, while those for univariable relationships for time-to-event efficacy endpoints were based on the log rank test for categorical independent variables and the likelihood ratio tests from Cox proportional hazard regression for continuous independent variables. Directionality is reported only for P values of ≤0.10. Directionality for the OC exposure variables is reported as follows: /, the drug effect was highest for the middle AUC<sub>0–24</sub> group compared to the low and high OC AUC<sub>0–24</sub> groups; U, the drug effect was lowest for the middle AUC<sub>0–24</sub> group compared to the low and high OC AUC<sub>0–24</sub> groups; \, the drug effect increased as OC AUC<sub>0–24</sub> increased; N, the drug effect decreased as OC AUC<sub>0–24</sub> increased; P<sub>0.001</sub>, the drug effect increased as IC<sub>50</sub> increased; P<sub>0.001</sub>, the drug effect was larger as the IC<sub>50</sub> decreased.

<sup>c</sup> Relationship directionality is based on time to 50% of the population achieving the endpoint.

### Table 3: Parameter or hazard ratio estimates for selected univariable models describing the relationship between continuous or time-to-event efficacy endpoints and OC AUC<sub>0–24</sub>

<table>
<thead>
<tr>
<th>Efficacy endpoint</th>
<th>Reference group for OC AUC&lt;sub&gt;0–24&lt;/sub&gt; (ng · h/ml)</th>
<th>Comparison group for OC AUC&lt;sub&gt;0–24&lt;/sub&gt; (ng · h/ml)</th>
<th>Univariable model&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Parameter estimate for continuous efficacy endpoint or hazard ratio estimate for time-to-event efficacy endpoint (95% CI)</th>
<th>Pairwise P value</th>
<th>Overall P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite symptom score AUC</td>
<td>≤1,495</td>
<td>&gt;1,495 to ≤14,180</td>
<td></td>
<td>−5.50 (−8.87, −2.31)</td>
<td>0.002</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>&gt;1,495</td>
<td>&gt;14,497</td>
<td></td>
<td>−7.88 (−12.42, −3.34)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;1,495 to 14,497</td>
<td>&gt;14,497</td>
<td></td>
<td>−2.36 (−6.44, 1.68)</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Time to alleviation of composite symptom score</td>
<td>≤1,568</td>
<td>&gt;1,568 to ≤13,638</td>
<td></td>
<td>1.94 (1.08, 3.50)</td>
<td>0.028</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>≤1,568</td>
<td>&gt;13,638</td>
<td></td>
<td>5.87 (2.71, 12.73)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;1,568 to 13,638</td>
<td>&gt;13,638</td>
<td></td>
<td>3.03 (1.51, 6.06)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Time to cessation of viral shedding</td>
<td>0</td>
<td>&gt;0 to ≤14,180</td>
<td></td>
<td>1.77 (1.01, 3.10)</td>
<td>0.048</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>&gt;14,180</td>
<td></td>
<td>2.85 (1.46, 5.57)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt;0 to 14,180</td>
<td>&gt;14,180</td>
<td></td>
<td>1.62 (0.941, 2.77)</td>
<td>0.082</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Parameter or hazard ratio estimates for selected univariable models describing the relationship between continuous or time-to-event efficacy endpoints and OC AUC<sub>0–24</sub> evaluated as a 3-group variable.

<sup>b</sup> All possible pairwise comparisons were evaluated.

<sup>c</sup> 95% CI, 95% confidence interval.
TABLE 4 Influence of OC AUC_{0–24} subgroups on the time to 25, 50, and 75% of the population achieving the continuous or time-to-event efficacy endpoint

<table>
<thead>
<tr>
<th>Efficacy endpoint</th>
<th>Subgroup threshold for OC AUC_{0–24} evaluated as a 3-group variable (ng · h/ml)</th>
<th>No. of subjects</th>
<th>Time to 25, 50, and 75% of the population achieving time-to-event dependent variable (days) or mean value for continuous variable*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite symptom score AUC</td>
<td>≤1,495, &gt;1,495 to ≤14,497, &gt;14,497</td>
<td>30 (29 placebo) 64 18</td>
<td>14.6 9.1 6.7</td>
</tr>
<tr>
<td>Time to alleviation of composite symptom score</td>
<td>≤1,568, &gt;1,568 to ≤13,638, &gt;13,638</td>
<td>20 (18 placebo) 30 14</td>
<td>2.5 3.5 4.5 1.5 0.5 1.5</td>
</tr>
<tr>
<td>Time to cessation of viral shedding</td>
<td>0, &gt;0 to ≤14,180, &gt;14,180</td>
<td>22 (22 placebo) 49 21</td>
<td>2.5 4.75 7 1.25 5.5 2.3</td>
</tr>
</tbody>
</table>

*All possible pairwise comparisons were evaluated.

guishable (e.g., 458.6 versus 459.8 for composite symptom score and 603.8 versus 604.3 for time to cessation of viral shedding), a final multivariable model was selected based upon consideration of which model was more informative of the nature of the exposure-response relationship (e.g., the model evaluating AUC_{0–24} as a 3-group variable would take precedence over that evaluating AUC_{0–24} as a 2-group variable). The multivariable models that were considered to be the final and hence most informative are represented by the bold P and AICc values.

While exposure-response relationships were apparent for composite symptom score AUC, time to alleviation of composite symptom score, and time to cessation of viral shedding, such relationships were not apparent for viral titer AUC and weak for peak viral titer endpoints. Specifically, relationships between viral titer AUC and OC AUC_{0–24} evaluated as a 2- or 3-group categorical variable were due to the contrasts between placebo- versus drug-receiving subjects, while the relationship with OC AUC_{0–24} as a quartile was N shaped. The relationship between peak viral titer and OC AUC_{0–24} evaluated as a 2-group categorical variable was only marginally significant (P = 0.049), while that for OC AUC_{0–24} evaluated as a 3-group categorical variable was ∩ shaped; no discernible association between peak viral titer and OC AUC_{0–24} evaluated as a continuous or quartile variable was evident. Thus, further consideration was not given to the multivariable models for viral titer AUC and peak viral titer.

The combination of simple plots and stratified Kaplan-Meier curves for subject cohorts defined by independent variables based on the above-described final multivariable models is shown in Fig. 3. The graphic illustrations provided in Fig. 3 demonstrate the influence of OC AUC_{0–24} group and IC_{50} on composite symptom score AUC (Fig. 3A) and time to alleviation of composite symptom score (Fig. 3B) and time to cessation of viral shedding (Fig. 3C).

Evaluation of the interactions between OC AUC_{0–24} group and IC_{50} failed to show any significance for each of the three models described above. As shown by the comparison of composite symptom score AUC among the six cohorts of subjects defined by two IC_{50} and three OC AUC_{0–24} groups in Fig. 3A, the lack of influence of IC_{50} was evident by the same pattern of improved efficacy among subjects with higher OC AUC_{0–24} values. Similarly, as shown by the Kaplan-Meier curves for six cohorts of subjects defined by the two IC_{50} and three OC AUC_{0–24} groups in Fig. 3B, earlier times to alleviation of composite symptom score were evident among subjects in higher OC AUC_{0–24} groups, irrespective of the IC_{50}. Last, as shown by the Kaplan-Meier curves for the six cohorts of subjects defined by the two IC_{50} and three OC AUC_{0–24} groups in Fig. 3C, earlier times to cessation of viral shedding were also evident among subjects in higher OC AUC_{0–24} groups, irrespective of the IC_{50} value.

DISCUSSION

The results of the analyses described herein represent the first robust exploration of exposure-response relationships for efficacy for an anti-influenza NAI using clinical data. Prior development of a population PK model enabled OC exposures to be determined for all subjects across two phase 2 studies, regardless of whether sparse or intensive PK samples were collected.

Despite the broad dose range in the two phase 2 studies, the limited number of schedules (i.e., dosing intervals) studied led to highly correlated exposure measures. Thus, it was not possible to evaluate which exposure, AUC_{0–24}, C_{min}, or C_{max} was most associated with efficacy. Given the preclinical findings derived from HFIM investigations of NAI (3, 4), AUC_{0–24} was chosen for evaluation in the PK-PD analyses carried out.

A two-stage evaluation for exposure-response relationships was then performed. The first stage involved the conduct of univariable analyses, with the primary objective to examine the relationship between each efficacy endpoint and OC AUC_{0–24}. The second stage of evaluation included multivariable analyses, for which the impact of IC_{50} (which also represented “study” or “influenza strain”) on each efficacy endpoint was also considered.

The key findings of the univariable analyses suggested the existence of biologically plausible and highly statistically significant
relationships between OC AUC_{0–24} evaluated as a 3-group variable with three efficacy endpoints. The optimally determined thresholds for the analyses based on evaluation of the 3-group OC AUC_{0–24} were, as might be expected, closely aligned for the clinical endpoints, composite symptom score AUC, and time to alleviation of composite symptom score (\(1,500\) and \(14,000\) ng · h/ml). The upper OC AUC_{0–24} threshold of 14,000 ng · h/ml was also closely similar to that for time to cessation of viral shedding, thus suggesting that the exposures required for maximal antiviral effect correlate with those required for maximal clinical outcome. Such findings are expected, given that NAI like OC, have highly specific antiviral activity.

Exposure-response relationships were not readily apparent for either peak viral titer or viral titer AUC endpoints. In retrospect, this was not unexpected based on the inoculation study design, as maximal virus titers following inoculation occurred early in therapy and are not primarily a function of drug exposure. Unlike time to cessation of viral shedding, the viral titer AUC is heavily influenced by peak virus titer. Thus, both peak virus titer and viral titer AUC are suboptimal efficacy endpoints for PK-PD evaluations when using data from an influenza inoculation study.

The observation that AUC_{0–24} beyond \(14,000\) ng · h/ml is associated with greater efficacy supports the rationale for investigating whether doses higher than the approved oseltamivir 75-mg BID dosing regimen, the average AUC_{0–24} for which is \(6,000\) ng · h/ml, will provide added benefit (2). This finding was consistent with observations from a small study comparing two different oseltamivir dosing regimens, 75 mg BID and 225 mg BID, in patients infected with pandemic H1N1 (pH1N1), from which provisional results suggest cessation of viral shedding at day 5 occurred in 75% of subjects for the higher dose versus 12% for the standard dose (\(P = 0.012\)) (M. A. Kumar, personal communication).

The findings described above are in contrast to the outcomes of two other studies in which 75 mg oseltamivir BID and 150 mg oseltamivir BID were compared in patients with seasonal influenza (15, 16) and findings from another study evaluating approved versus double dosing of oseltamivir in avian and severe

![FIG 2 Univariable relationships between composite symptom score (A), time to alleviation of composite symptom score (B), and time to cessation of viral shedding (C) and OC AUC_{0–24} (ng · h/ml) evaluated as a 3-group variable. ANOVA, analysis of variance.](http://aac.asm.org/attachment/figure2.png)
influenza (17). These three studies failed to demonstrate superiority of the higher dose versus the approved regimen. However, these studies were not designed to test whether exposure-response relationships existed beyond any apparent lack of a dose-response relationship. We also suspect, that by virtue of the study designs, the resultant PK exposures in patients in each dose group may not have been sufficiently different given only a 2-fold difference in doses investigated. Thus, this may have effectively reduced the “power” of a study to detect a difference in efficacy by dose and may be the basis for why Kumar et al. appear to have demonstrated a difference between doses with fewer patients but a greater consistency of model estimates with increasing efficacy endpoints, composite symptom score, and time to cessation of viral shedding, were those for which OC AUC$_{0–24}$ was evaluated as a 3-group variable. It was noted that some of the pairwise comparisons between OC AUC$_{0–24}$ groups were nonsignificant. Nonetheless, given the arrays of the significant comparisons across all the endpoints, the consistency of model estimates with increasing efficacy, and especially, the significant differences across each pairwise comparison for time to alleviation of composite symptom score, the available evidence is supportive of the existence of an exposure-response relationship for efficacy of oseltamivir.

Among the above-described final multivariable models, no strong evidence was seen that would suggest IC$_{50}$ (i.e., a surrogate for study name or virus type) had influence on efficacy endpoints. This was an unexpected finding even in the inoculation study setting, as it suggests that the AUC$_{0–24}$ thresholds were not im-

### TABLE 5 Summary of $P$ values and directional assessments for multivariable relationships between efficacy endpoints and OC AUC$_{0–24}$

<table>
<thead>
<tr>
<th>Efficacy endpoint</th>
<th>Directionality and $P$ value or AICc for the form of OC AUC$_{0–24}$ evaluated$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite symptom score AUC</td>
<td>$\langle &lt;0.001; 458.6/0.003 \rangle$, 459.8/0.08, 466.8/0.029, 465.1</td>
</tr>
<tr>
<td>Viral titer AUC</td>
<td>$\langle 0.004; 342.0/0.009 \rangle$, 342.5/N, 0.042, 345.5/0.06, 346.9</td>
</tr>
<tr>
<td>Peak viral titer</td>
<td>$\langle 0.033; 99.1/0.022 \rangle$, 97.6/0.12, 101.2/0.15, 101.6</td>
</tr>
<tr>
<td>Time to alleviation of composite symptom score</td>
<td>$\langle &lt;0.001; 381.9/0.001 \rangle$, 379.6/0.036, 388.4/0.0002, 384.1</td>
</tr>
<tr>
<td>Time to cessation of viral shedding</td>
<td>$\langle 0.026; 603.8/0.033 \rangle$, 604.3/0.13, 607.4/0.11, 606.8</td>
</tr>
</tbody>
</table>

$^a$ Summary of $P$ values and directional assessments for multivariable relationships between efficacy endpoints and OC AUC$_{0–24}$ evaluated as a continuous, 2-group, 3-group, and quartile categorical variables, adjusted for IC$_{50}$.

$^b$ As described in Materials and Methods for multivariable analyses, $P$ values for multivariable relationships for continuous efficacy endpoints were based on the Wald test for single parameters (i.e., 2-group and continuous forms of an independent variable) and likelihood ratio test for multiple parameters (3-group and quartile forms of an independent variable). Directionality is reported only for $P$ values of $\leq 0.10$. Directionality for the OC AUC$_{0–24}$ variables is reported as follows: $\langle$, the drug effect was highest for the middle OC AUC$_{0–24}$ group compared to the low and high OC AUC$_{0–24}$ groups; $>$, the drug effect increased as OC AUC$_{0–24}$ increased; N, the drug effect was lowest with the first and third OC AUC$_{0–24}$ quartiles and highest with the second and fourth OC AUC$_{0–24}$ quartiles. The relationships between the efficacy endpoints and OC AUC$_{0–24}$ have been adjusted for IC$_{50}$.

$^c$ The multivariable models that were considered to be the most informative and thus, final, are represented by the bold $P$ and AICc values.

### TABLE 6 Summary of the final multivariable models for continuous or time-to-event efficacy endpoints containing OC AUC$_{0–24}$ evaluated as a 3-group variable$^a$

<table>
<thead>
<tr>
<th>Efficacy endpoint</th>
<th>Independent variable$^b$</th>
<th>Reference group</th>
<th>Comparison group</th>
<th>Parameter estimate for continuous efficacy endpoint or hazard ratio estimate for time-to-event efficacy endpoint (95% CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite symptom score AUC</td>
<td>IC$<em>{50}$ (nM) AUC$</em>{0–24}$ (ng · h/ml)</td>
<td>0.18 14,95 14,97</td>
<td>$&gt;14,97$ $&gt;14,97$ $&gt;14,97$</td>
<td>1.77 (−1.31, 4.85) $&gt;5.36$ (−8.73, −1.99) $&gt;7.03$ (−11.8, −2.27) $&gt;1.68$ (−5.91, 2.56)</td>
<td>0.257 0.0021 0.0042 0.434</td>
</tr>
<tr>
<td>Time to alleviation of composite symptom score</td>
<td>IC$<em>{50}$ (nM) AUC$</em>{0–24}$ (ng · h/ml)</td>
<td>0.18 1,568 1,568</td>
<td>$&gt;1,568$ $&gt;1,568$ $&gt;1,568$</td>
<td>1.76 (0.46, 1.45) 1.85 (1.01, 3.39) 5.34 (2.37, 12.07) $&lt;0.001$</td>
<td>0.494 0.045 0.0003</td>
</tr>
<tr>
<td>Time to cessation of viral shedding</td>
<td>IC$<em>{50}$ (nM) AUC$</em>{0–24}$ (ng · h/ml)</td>
<td>0.18 0 0</td>
<td>$&gt;0.14,180$ $&gt;0.14,180$ $&gt;0.14,180$</td>
<td>0.66 (0.38, 1.14) 1.72 (0.98, 3.03) 2.42 (1.20, 4.84)</td>
<td>0.136 0.059 0.013</td>
</tr>
</tbody>
</table>

$^a$ Summary of the final multivariable linear regression or Cox regression for continuous or time-to-event efficacy endpoints containing OC AUC$_{0–24}$ evaluated as a 3-group variable.

$^b$ Pairwise comparisons were made between the comparison and reference groups.
pacted by influenza A/Texas versus influenza B/Yamagata or the \sim 100\text{-}fold range difference between the \(\text{IC}_{50}\) values (0.18 to 16.76 \(\mu\text{M}\)). Further exploration in natural influenza infection is needed to explore the impact of \(\text{IC}_{50}\) and influenza virus strain and subtype on the OC exposure-response relationships for efficacy.

There are several limitations to the study data utilized for the analyses described herein. These analyses represent a retrospective pharmacometric assessment of pooled data from phase 2 inoculation studies. Data were from experimental pharmacology studies and may not represent efficacy in natural influenza infection. Nevertheless, the highly controlled nature of these types of studies enhances the ability to explore exposure-response relationships and generate hypotheses to optimize dosing regimens for further study in the setting of natural influenza.

The results of these analyses, which utilized data from healthy subjects in two phase 2 inoculation studies, provided the first demonstration of the existence of exposure-response relationships for the efficacy of oseltamivir against influenza. These findings also suggest that OC exposures beyond those that are achieved with the approved oseltamivir dosing regimen will provide enhanced efficacy. The con-

FIG 3 Composite symptom score AUC (A), time to alleviation of composite symptom score (B), and time to cessation of viral shedding (C) by subject cohorts defined by independent variables based on the final multivariable models containing OC AUC\(0 – 24\) (ng \cdot h/ml) evaluated as a 3-group variable.
founded factors of this study, influenza strain and IC50 did not influence efficacy endpoints. The clinical applicability of these observations requires further investigation but may have important implications for the future management of influenza.

REFERENCES


14. Reference deleted.


Population Pharmacokinetics of Oseltamivir: Pediatrics through Geriatrics


Hoffmann La-Roche, Inc., Nutley, New Jersey, USA; Institute for Clinical Pharmacodynamics, Latham, New York, USA; d3 Limited, Hong Kong, Hong Kong; Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, Australia; University at Buffalo, Buffalo, New York, USA; University of Oxford, Oxford, United Kingdom

Oseltamivir is a potent inhibitor of influenza virus neuraminidase enzymes essential for viral replication. This study aimed to investigate the impact of covariates on pharmacokinetic (PK) variability of oseltamivir and its active metabolite form, oseltamivir carboxylate (OC). Dosing history, plasma drug concentrations, and demographic information were pooled from 13 clinical trials providing data for 390 healthy and infected subjects ranging in age from 1 to 78 years and given oseltamivir doses of 20 to 1,000 mg. Candidate population PK models simultaneously characterizing the time course of oseltamivir and OC in plasma were evaluated by using the NONMEM software program, and subject covariates were assessed using stepwise forward selection (α = 0.01) and backward elimination (α = 0.001). A two-compartment model with first-order absorption of oseltamivir and first-order conversion of oseltamivir to OC and a one-compartment model with first-order elimination of OC were utilized. Body weight when evaluated using a power function was a significant predictor of the apparent oseltamivir clearance and both apparent OC clearance (CL/F) and central volume of distribution (VC/F). Creatinine clearance was a significant predictor of CL/F, while VC/F also decreased linearly with age. A visual predictive check indicated that the final model described oseltamivir and OC concentrations in plasma adequately across dose regimens and subject covariate ranges. Concordance of population mean and individual post hoc predictions of maximum concentration of drug at steady state (Cmax) and area under the plasma drug concentration–time curve from 0 to 24 h at steady state (AUC0–24) was high (r² = 0.81 and 0.71, respectively). In conclusion, a comprehensive population PK model was constructed to bridge the adult to pediatric oseltamivir PK data, allowing for reasonable estimation of the PK of OC using subject demographic data alone.

Oseltamivir is a potent antiviral medication that selectively inhibits influenza virus neuraminidase enzymes essential for efficient release of intact virions from an infected cell to allow further infection of cells in vivo. Oseltamivir has been shown to be safe and effective and is used worldwide for the treatment and prophylaxis of seasonal and pandemic influenza (1–6). Oseltamivir is orally administered as an inactive ethyl ester prodrug which undergoes rapid conversion by hepatic esterases into its active metabolite form, oseltamivir carboxylate (OC) (7, 8).

The pharmacokinetic (PK) properties of both the prodrug and OC have been well studied (7). Approximately 80% of an oral dose of the prodrug is converted into OC by hepatic metabolism via carboxylesterase 1A1 (HCE1), with less than 5% of prodrug recovered unchanged in the urine. The rate-limiting step for the appearance of OC in plasma is the release of the formed OC metabolite from the hepatocyte (8, 9). Once in circulation, OC is predominately cleared by the kidney via glomerular filtration and renal secretion (8, 9). OC distributes moderately into tissues having an absolute volume of distribution that approximates total body water (25 to 45 liters). Following oral dosing, plasma oseltamivir concentrations decline rapidly with an apparent elimination half-life (t1/2) of 1 to 3 h, while OC has a t1/2 of 6 to 10 h (7).

Clinical pharmacokinetic-pharmacodynamic (PK-PD) indices for efficacy and resistance are currently being investigated for oseltamivir in both adult and pediatric populations. One major challenge underlying such PK-PD analyses is that a significant portion of the infected patients who participated in clinical trials involving oseltamivir have available efficacy data (e.g., viral load measurements, symptom scores, and the time to resolution of symptoms) but either little or no measured plasma PK data available. The objectives of this analysis were twofold: (i) to develop a parsimonious population PK model for the oseltamivir prodrug and its active OC metabolite using all PK data collected thus far in adult and pediatric subjects receiving the currently marketed oral oseltamivir formulation and (ii) to identify and assess the impact of subject covariates on oseltamivir PK variability. This population PK model may be used to determine the time course of OC in both pediatric and adult subjects in order to further assess PK-PD relationships for efficacy and ultimately may help guide dosing recommendations for renal insufficiency or other special populations. In cases where oseltamivir PK data were not collected, such a model may potentially be used to estimate the time course of OC in plasma simply by using subject demographic data, thereby expanding the size of the PK-PD evaluable population.

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doi:10.1128/AAC.02438-12
TABLE 1 Studies utilized in the oseltamivir population PK analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Subject type</th>
<th>Study design and oseltamivir dose</th>
<th>PK sampling scheme</th>
<th>Total no. of subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>WP15517</td>
<td>Healthy adults</td>
<td>Single-center, double-blind, single ascending dose study. Placebo, 20, 50, 100, 200, 500, and 1,000 mg were administered orally</td>
<td>At 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 18, 24, 33, and 48 h after dose</td>
<td>36</td>
</tr>
<tr>
<td>WP15525</td>
<td>Healthy adults</td>
<td>Single-center, double-blind, multiple ascending dose study. Placebo, 50-, 100-, 200-, and 500-mg oral doses administered BID for 7 days</td>
<td>Day 1 at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, and 12 h; days 3, 5, and 7 before the morning dose; day 7 at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 18, and 24 h after the last dose</td>
<td>24</td>
</tr>
<tr>
<td>PV15616</td>
<td>Healthy adults, inoculated with influenza virus A</td>
<td>Single-center, double-blind study. Placebo, 20, 100, 200 mg oral BID, or 200 mg oral qd. Dosing initiated 28 h after virus inoculation and continuing for 5 days</td>
<td>Days 3, day 4, and day 7 prior to the morning dose</td>
<td>64</td>
</tr>
<tr>
<td>NP15717</td>
<td>Healthy adults, inoculated with influenza virus B</td>
<td>Single-center, double-blind study. Placebo, 75, or 150 mg administered orally BID for 5 days</td>
<td>Day 1 and day 5 at predose, 0.5, 1, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0, and 12.0 h. Predose samples on days 2 and 4</td>
<td>40</td>
</tr>
<tr>
<td>WV15670</td>
<td>Adult patients, influenza infected</td>
<td>Multicenter, double-blind study. Placebo, 75, or 150 mg administered orally BID for 5 days</td>
<td>Day 5 at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 h after the morning dose</td>
<td>30</td>
</tr>
<tr>
<td>WV15671</td>
<td>Adult patients, influenza infected</td>
<td>Multicenter double-blind study. Placebo, 75, or 150 mg administered orally BID for 5 days</td>
<td>Day 5 at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 h after the morning dose</td>
<td>9</td>
</tr>
<tr>
<td>WV15730</td>
<td>Adult patients, influenza infected</td>
<td>Multicenter, double-blind study of two parallel groups. Placebo or 75 mg BID administered orally for 5 days</td>
<td>Day 5 at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 h after the morning dose</td>
<td>5</td>
</tr>
<tr>
<td>WP15647</td>
<td>Healthy geriatric subjects (ages ≥ 65 yrs)</td>
<td>Single-center, double-blind study. A single 150-mg oral dose or placebo</td>
<td>Days 1 and 7 predose and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10, 12, 18, and 24 h after dose</td>
<td>18</td>
</tr>
<tr>
<td>WP15648</td>
<td>Renally impaired adults</td>
<td>Single-center, open-label study. A 100-mg oral dose on day 1 followed by BID dosing on days 2 to 5 and a single dose on day 6 administered to healthy and mildly, moderately, and severely renally impaired subjects</td>
<td>Days 1 and 6 at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, and 24 h after dose. Day 2.1 h before dose. Day 4.1 h before dose and 72 h. Day 6 at 120 and 132 h</td>
<td>14</td>
</tr>
<tr>
<td>WV15758</td>
<td>Pediatric patients, influenza infected (ages 1 to 12 yrs)</td>
<td>Multicenter, double-blind placebo-controlled efficacy study in children with doses of 2 mg/kg or placebo administered orally (suspension) BID for 5 days</td>
<td>At all sites, 2 predose troughs and 1 peak 2 to 4 h after dosing. Samples taken during 2 visits at least 24 h after start of therapy. At selected sites, full profile taken predose and at 1, 2, 3, 4, 6, 8, and 12 h after dosing obtained any time during treatment</td>
<td>90</td>
</tr>
<tr>
<td>NP15826</td>
<td>Pediatric and adolescent patients, influenza infected (ages 5 to 18 yrs)</td>
<td>Single-center, open-label, single-dose safety and PK study with 3 age groups, 5 to 8, 9 to 13, and 14 to 18 yrs old, each receiving 2-mg/kg oral suspension</td>
<td>At 0.5, 1, 2, 3, 4, 6, 8 10, 12, and 24 h after dose</td>
<td>18</td>
</tr>
<tr>
<td>JV16284</td>
<td>Pediatric patients, influenza infected (ages 1 to 12 yrs)</td>
<td>Multicenter, open-label, multiple-dose study with 2-mg/kg oral suspension administered BID for 5 days</td>
<td>At 4 h and 12 h after a dose at more than 48 h after the start of therapy initiation</td>
<td>18</td>
</tr>
<tr>
<td>PP16351</td>
<td>Healthy pediatric subjects (ages 1 to 5 yrs)</td>
<td>Single-center, open-label, single-dose study with 30 mg (1 to 2 yrs) and 45 mg (3 to 5 yrs) administered as an oral suspension</td>
<td>At 1, 2, 3, 4, 8, 12, and 24 h after dose</td>
<td>24</td>
</tr>
</tbody>
</table>

* BID, twice a day; qd, once a day.

Materials and Methods

Data. Data from 390 subjects, who received various oral doses of oseltamivir ranging from 20 to 1,000 mg as either a single-dose or repeated-dose regimen in 13 clinical studies and for whom PK data were available were used to develop the oseltamivir population PK model (Table 1). All studies were performed in accordance with the Declaration of Helsinki. Adult subjects provided written, informed consent before entering the studies, with parents or legal guardians providing consent on behalf of the child where necessary, and the relevant study protocols were approved by the institutional review board at each study site. The database was comprised of a renal impairment study in adults (WP15648) (8), two phase 1 healthy adult single- and multiple-dose studies (WP15517 and WP15525) (10),
two phase 2 adult influenza inoculation studies (PV15616 and NP15717) (5, 11), three adult phase 3 studies for the treatment of acute influenza (WV15670, WV15671, and WV15730) (3, 4), four pediatric studies (WV15758, PP16351, JV16284, and NP15826) (12, 13), and a geriatric study (WP15647) (8). These studies represent all of the PK data available for subjects given the currently marketed oseltamivir formulation with the exception of data obtained from drug–drug interaction studies, from end-stage renal disease patients undergoing intermittent hemodialysis, and from a recent study conducted in neonates and infants (<1 year of age) which was not available at the time this analysis was performed.

Intensive PK sampling (>5 samples/subject) was performed in 10 of the 13 clinical studies as summarized in Table 1. Generally, across all 10 studies, intensive sampling occurred at 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10, and 12 h after the morning dose. A sparse PK sampling strategy was employed in the adult inoculation study PV15616 (3 samples/subject) and pediatric studies JV16284 (2 samples/subject) and WV15758 (3 samples/subject). In study WV15758, selected sites collected a full PK profile (>7 samples/subject) for 5 pediatric patients.

The bioanalytical procedure for determination of plasma oseltamivir and OC concentrations was described previously by Rayner et al. (10). Oseltamivir and OC concentrations were determined using a validated method in which high-performance liquid chromatography is coupled to tandem mass spectrometry (HPLC/MS/MS). The assay method was sufficiently sensitive, with a lower limit of quantification (LLOQ) of ≥1 ng/ml for the oseltamivir prodrug and ≥8.8 ng/ml for the OC metabolite. The method was also accurate and precise, providing overall coefficient of variations (CV) of ≤3% and ≤6%, respectively. Only 3 plasma oseltamivir concentrations that were reported as not quantifiable were excluded from analysis. Because of the significantly shorter half-life of the prodrug relative to the metabolite, it should be noted that not all blood samples collected at later times were analyzed for prodrug concentrations due to cost considerations.

Structural model development. A parsimonious structural population PK model describing the time course of oseltamivir and OC in plasma, which was data driven and fit for the purpose of investigating the impact of subject covariates on oseltamivir and OC PK variability, was constructed using the ADVAN 13 subroutine in the nonlinear mixed-effects modeling software NONMEM version 7, level 1.2 (ICON Development Solutions, Endicott City, MD) using the Intel Visual Fortran Compiler Professional, version 11.1.035. The first-order conditional estimation method with interaction was used for parameter estimation. With such a low incidence of data below the LLOQ, there was no perceived benefit to moving toward a more time-consuming and computationally complex likelihood-based methodology (e.g., Beal M3 approach which utilizes the LAPLACIAN estimation method in NONMEM). Data set creation and postprocessing of the NONMEM output were performed using SAS version 9.2 (SAS Institute, Cary, NC).

Oseltamivir and OC PK data were modeled simultaneously in this analysis. During evaluation of candidate structural PK models, a two-compartment model was tested for the oseltamivir prodrug, and both one- and two-compartment models were tested for the OC metabolite based upon inspection of the individual concentration–time profiles. The models were parameterized using a first-order absorption rate constant (k a), apparent clearance for both oseltamivir (CL/F) and OC (CL/F), central volume of distribution for both oseltamivir (V d/F) and OC (V d/F), and a distribution clearance (CLd/F) and volume of distribution term (Vp/F) for each peripheral compartment added. All oseltamivir volume and clearance terms are conditioned on the fraction of the prodrug absorbed, and the OC PK parameters were additionally conditioned on the fraction of the prodrug metabolized to OC.

Candidate models were evaluated using the following criteria: examination of the population mean PK parameter estimates and their precision as measured as percent standard error of the mean (%SEM), graphical examination of standard population-based diagnostic goodness-of-fit plots, graphical examination of the observed versus individual post hoc predicted concentration–time profiles, assessing the magnitude of both interindividual and residual variability, and assessing changes in the minimum value of the objective function (MVOF). For hierarchical models, a decrease in the MVOF of at least 10.8 upon addition of a parameter was considered statistically significant (P < 0.001, 1 degree of freedom) according to a chi-square distribution.

Unexplained interindividual variability in PK model parameters was estimated using an exponential model with the random effect 𝜷 as follows: 𝜷 = TVP × exp(𝜷). In this model, TVP is the population mean PK parameter in the population (typical value in the population), 𝜷 is the individual post hoc estimate for the PK parameter in the jth subject, and 𝜷 is a normally distributed random variable with a mean of zero and a variance 𝜷. This model assumes that the likelihood distribution for the PK parameter is log normal.

Separate additive plus constant coefficient of variation (CCV) error models were used to describe random residual variability for the plasma oseltamivir and OC concentrations as follows: Cij = Cij* + Cij* × 𝜷CCV + 𝜷ADD. In this model, 𝜷 is the jth plasma concentration measured in the jth subject and 𝜷* is the model predicted concentration, 𝜷CCV and 𝜷ADD are the CCV and additive error terms, respectively, which are normally distributed random variables with a mean of zero and variance 𝜷CCV and 𝜷ADD.

Covariate evaluation. Subject covariates evaluated included age (in years), weight (in kilograms), height (in centimeters), gender, race, body surface area (BSA) (in m²), serum creatinine (SCr) (in milligrams per deciliter), and creatinine clearance (Ccr) (in ml/min/1.73 m²). In adults of ≥18 years of age, Clcr was calculated using the Cockcroft and Gault equation (14), after substituting ideal body weight (IBW) (15) for weight if the weight was greater than IBW and after normalizing to a BSA of 1.73 m². In pediatric and adolescent subjects 1 to 17 years of age, Clcr was instead calculated as 0.413HTCM/SCr where HTCM is the height in centimeters using the revised Schwartz (16) equation. In each method, SCr was capped at a lower bound of 0.7 mg/dl (adult subjects) or 0.2 mg/dl (pediatric and adolescent subjects) to prevent calculation of Clcr values that exceed normal physiological expectations and thus not likely reflective of the glomerular filtration rate (GFR). Since there is no physiological or pharmacological basis for expecting any PK differences between healthy subjects and patients with seasonal influenza and no such difference has previously been reported (2), the presence of infection was not tested as a covariate, since differences in age, body size, and renal function would confound this assessment.

The ability of these subject covariates to explain a portion of the PK variability for oseltamivir and OC was evaluated using stepwise univariate forward selection (α = 0.01) followed by backward elimination (α = 0.001) procedures. To explore the shape and magnitude of the covariate-parameter relationships in each step of forward selection, the random effects expressed as the difference between the population mean and individual post hoc estimate of the PK parameter were plotted against each covariate (delta plots). Continuous covariate-parameter relationships showing a graphical trend were formally tested univariately within NONMEM using either a linear or power function centered on the median of the covariate. An exception to this general rule was made when evaluating weight, where 70 kg was utilized to allow for a more convenient comparison of the estimated parameters to a typical adult subject. Categorical subject covariates were assessed as a proportional shift in the typical population mean value. In each step of forward selection, the covariate effect resulting in the largest significant decrease in the MVOF was retained in the population PK model, and the delta plots were reevaluated to verify that the covariate effect was properly accounted for in the model. In each step of backward elimination, the covariate effect resulting in the smallest nonsignificant increase in the MVOF was removed from the population PK model.

Model evaluation. After completing the covariate analysis, delta plots were generated separately for infected versus noninfected subjects using the final model to verify that there were no biases or observable differences.
between these populations which need to be accounted for in the model. To investigate the clinical impact of covariates included in the final population PK model, population mean predicted plasma OC concentration-time profiles for typical subjects administered the standard influenza treatment regimen of 75 mg every 12 h (q12h) for 5 days were generated while allowing the covariate of interest to vary and fixing the other covariate effects.

A prediction-corrected visual predictive check (PC-VPC) was also performed to assess the appropriateness of both the fixed and random effects for the final population PK model by graphically examining the agreement between the 5th, 50th, and 95th percentiles of the observed and simulation mean predicted OC concentration-time data for the same number of patients in 250 new simulation data sets created using the NSUBPROBLEMS = 250 option for $SIMULATION$ within NONMEM. Prediction correction was used to adjust for the differences within a time interval coming from independent variables in the population PK model, allowing all of the data to be plotted together to avoid stratification by factors such as time (e.g., single versus repeated dosing), dose amount, or subject covariate effects.

In addition, the concordance between individual post hoc and population mean predicted OC $C_{\text{max}}$ (maximum OC concentration at steady state) and AUC$_{0–24}$ (area under the plasma OC concentration-time curve from 0 to 24 h at steady state) was graphically assessed to investigate the suitability of the final population PK model for predicting OC exposure metrics and concentration-time profiles in subjects with demographic data but no measured PK. To accomplish this, both the population mean and individual post hoc parameters were used to output plasma OC concentrations every 0.1 h for each subject in the analysis data set during the 12- or 24-h dosing interval after 5 days of therapy. $C_{\text{max}}$ was calculated using direct observation, while AUC$_{0–24}$ was calculated via numerical integration. A coefficient of determination ($r^2$) of at least 0.7 for a particular metric was taken to indicate reasonably acceptable concordance between the individual post hoc and population mean predicted OC exposures.

RESULTS

The analysis data set included a total of 3,881 oseltamivir concentrations and 4,402 OC concentrations. No outlier observations were detected. Of the 390 subjects included in the analysis, 241 were males and 149 were females. The median age was 21 years (range, 1 to 78 years), the median weight was 64.5 kg (range, 8 to 115 kg), and the median CL$_{\text{CR}}$ was 95.1 ml/min/1.73 m$^2$ (range, 13.9 to 178 ml/min/1.73 m$^2$). There were a total of 297 subjects with normal renal function (CL$_{\text{CR}}$ of ≥80 ml/min/1.73 m$^2$), 73 subjects with mild renal dysfunction (CL$_{\text{CR}}$ of 50 to 80 ml/min/1.73 m$^2$), 19 subjects with moderate renal dysfunction (CL$_{\text{CR}}$ of 30 to 49 ml/min/1.73 m$^2$), and 1 subject with severe renal dysfunction (CL$_{\text{CR}}$ of <30 ml/min/1.73 m$^2$).

The most parsimonious model describing the time course of oseltamivir and OC in plasma consisted of two disposition compartments for oseltamivir, with direct conversion of oseltamivir to OC, and a single disposition compartment for OC (Fig. 1 and Table 2). A two-compartment model for the OC metabolite was also tested at this stage of the analysis, resulting in only a modest decrease in MVOF and no noticeable improvement in model diagnostic plots relative to the one-compartment model. Interindividual variability was estimated for all of the structural parameters ($k_o$, CL$_{\text{p/F}}$, V$_{c_p/F}$, CL$_{d/F}$, V$_{p/F}$, CL$_{\text{m/F}}$, and V$_{c_m/F}$). All parameters were estimated with good precision (SEM < 32%), and there

![FIG 1 Structural PK model for oseltamivir (OP) and oseltamivir carboxylate (OC).](image-url)
was between 30% and 70% interindividual variability across parameters. Modest correlations were detected between \( CL_p/F \) and \( CL_m/F \) (\( r^2 = 0.372 \)) and between \( V_c_p/F \) and \( V_c_m/F \) (\( r^2 = 0.274 \)) when estimated in the final population PK model, and both terms were statistically significant (\( P < 0.00001 \)). Residual variability for oseltamivir was best described using a reduced CCV error model and had a magnitude of 40.5% CV. The residual variability for plasma OC concentrations was described with the additive plus CCV error model, and the magnitude ranged from 49.8% to 14.3% CV for plasma OC concentrations ranging from 0.05 to 6 mg/liter.

Oseltamivir \( CL_p/F \) and OC \( CL_m/F \) and \( V_c_m/F \) increased asymptotically with weight as would be expected according to allometric principles. These covariate effects were modeled using power functions with estimated power exponents for weight on \( CL_p/F, CL_m/F, \) and \( V_c_m/F \) of 0.838, 0.560, and 0.830, respectively. OC volume also decreased linearly with age; however, this was a relatively minor covariate effect. The effect of weight on \( CL_p/F \) was the most statistically significant covariate effect (308-unit decrease in MVOF, \( P < 0.00001 \)) in the first step of the forward selection and helped reduce interindividual variability in \( CL_p/F \) from 70% to 42% CV from the base structural model to the final model. Inclusion of the effect of weight on \( CL_p/F \) (149-unit decrease in MVOF, \( P < 0.00001 \)) and \( V_c_m/F \) (62-unit decrease in MVOF, \( P < 0.00001 \)) in steps 2 and 3, as well as the relatively smaller effect of age on \( V_c_m/F \) (18-unit decrease in MVOF, \( P = 0.00002 \)) in step 4, collectively resulted in an additional 11% to 12% CV decrease in the interindividual variability for these parameters. The effect of \( CL_{CR} \) on \( CL_m/F \) became apparent in the forward selection process only after weight was accounted for in the model to adjust for body size. In the final model, there did not appear to be any differences in oseltamivir PK between infected and noninfected subjects.

In the final population PK model, there was good concordance between the observed OC concentrations and both the population mean predicted (\( r^2 = 0.741 \)) and individual post hoc predicted (\( r^2 = 0.969 \)) OC concentrations across subjects in all age groups (\( \leq 8 \) years, 9 to 17 years, 18 to 65 years, and \( > 65 \) years). The plots show random scatter around the line of identity with no systematic bias observed. The concordance between the observed concentrations and both the population predicted (\( r^2 = 0.620 \)) and individual predicted (\( r^2 = 0.765 \)) oseltamivir concentrations while not as high were also adequate, and plots of conditional weighted residuals versus time since the last dose for both oseltamivir and OC were unbiased (see the supplemental material).

For the prodrug, the \( \eta \)-shrinkage values were 46.9% for \( k_w \), 11.9% for \( CL_p/F \), 33.7% for \( V_c_p/F \), 23.4% for \( CL_d/F \), and 35.5% for \( V_d/F \). For the OC metabolite, the \( \eta \)-shrinkage values were 7.16% for \( CL_m/F \) and 11.4% for \( V_c_m/F \). These reasonably low \( \eta \)-shrinkage values confirm that diagnostics based on the individual post hoc parameter estimates can reliably be used to assess model fit. A PC-VPC (Fig. 3) was used to assess the predictive performance of the final population PK model. This plot demonstrated that there was acceptable agreement between the 90% confidence intervals for the (prediction-corrected) observed and simulated plasma oseltamivir and OC concentrations over time since the last dose when examined by age group. Collectively, these findings suggested that the final population PK model adequately described the data obtained from this population.

To further investigate the clinical impact of age, weight, and \( CL_{CR} \), the final population PK model was used to deterministically simulate population mean OC concentration-time profiles at steady state for typical adult subjects receiving the standard influenza treatment regimen of 75 mg q12h for 5 days. Figure 4A shows the impact of renal function on the PK profile of OC, with a typical 40-year-old subject weighing 70 kg and having a \( CL_{CR} \) of 30 ml/min/1.73 m² showing an approximate 2-fold increase in OC exposure relative to a subject with normal renal function (\( CL_{CR} \) of 120 ml/min/1.73 m²). Figure 4B illustrates the impact of weight on the PK profile of OC in adult subjects with normal renal function, where a 1.38-fold increase in the maximum OC exposure was predicted as weight increases from 50 to 90 kg for an 18-year-old subject. The impact of age was also demonstrated to be negligible as evident by the fact that the maximal OC exposure increased only 1.08- to 1.17-fold between an 18-year-old and 55-year-old subject across these same weights.

To investigate the suitability of the final model for prediction of OC exposure measures in subjects with demographic data but no measured PK, the concordance of individual post hoc predictions (population PK model including covariates after considering measured drug concentrations) and population mean predictions...
was assessed. As shown in Fig. 5, the concordance of population mean and individual post hoc predictions of $C_{\text{max}}$ and $AUC_{0-24}$ was acceptable ($r^2 = 0.807$ and 0.713, respectively).

**DISCUSSION**

A population approach was utilized to develop a parsimonious structural PK model for oseltamivir and its active OC metabolite which served as the basis for identifying the clinical determinants of oseltamivir PK variability. This analysis was conducted using a large multistudy database comprised of the majority of the plasma PK data collected thus far following oral administration of oseltamivir to pediatric, adult, and elderly subjects. Unlike earlier models developed for oseltamivir in healthy adult subjects (10) which attempted to better understand the first-pass effect of oseltamivir as well as the relative contributions of renal and nonrenal clearance, the goal of the current analysis was to derive a more-simplified model suitable for characterizing the intensive and/or sparse sampling plasma PK data for orally administered oseltamivir and its active OC metabolite in both pediatric and adult subjects.

During structural model development, a two-compartment PK model with first-order absorption and elimination best characterized the disposition of oseltamivir in plasma, featuring direct conversion of oseltamivir to OC. A one-compartment model with first-order elimination adequately characterized the disposition of OC. This structural population PK model does not explicitly model the presystemic first-pass metabolism of oseltamivir to OC, as previously had been done by Rayner et al. (10), given the desire to simplify the PK model by using only the plasma PK data obtained following oral administration of oseltamivir. This is an especially important consideration given that clinical trials of oral oseltamivir conducted in infected adult and pediatric subjects did not determine oseltamivir PK in urine. A similar simplified structural population PK model has been used previously by other investigators (18); however, that particular model attempted to...
estimate renal and nonrenal clearance without comodeling urinary PK data. Inherent in this PK modeling approach is that the PK parameters for oseltamivir are conditioned on the fractional absorption of oseltamivir from the gastrointestinal (GI) tract, since PK data following intravenous (i.v.) administration was not included in this analysis. Since oseltamivir is predominantly converted to OC with only a small fraction (~5%) eliminated renally as the unchanged prodrug, it was assumed that oseltamivir is completely converted to the OC metabolite and subsequently the PK parameters for OC are also conditioned on the fraction of circulating prodrug metabolized to OC.

Prior to conducting the covariate analysis, both allometric and empirical approaches as described elsewhere (19) were considered to bridge the adult PK data to the pediatric PK data in order to account for body size and maturation differences on oseltamivir PK. The allometric approach includes the effect of weight a priori on each PK parameter using a power function before assessing the impact of other covariates. To aid with interpretation, the coefficient term is centered around a 70-kg adult and the power term is estimated to be 519 liters/h for a 70-kg adult, and the estimated exponent of 0.84 approximated the standard allometric value of 0.75 (19). Oseltamivir CL/F is predominantly due to hepatic conversion to OC by carboxylesterase 1A1 (HCE1) (8). HCE1 expression has been shown to surge during the postneonatal period, allowing infants to rapidly achieve nearly half of the hydrolytic activity of an adult (20). Additional in vitro data have suggested that HCE1 expression in liver samples derived from children 1 to 10 years of age may also be slightly lower than in adults (21, 22). Despite these age-related differences in HCE1 function, oseltamivir is a high extraction drug, and thus, CL/F is primarily dictated by hepatic blood flow which normalizes to adult values shortly after birth (23). This may explain why age was not a significant predictor of oseltamivir CL/F in this analysis (in pediatric subjects of >1 year of age) once body weight is accounted for in the model to account for capacity and hepatic blood flow differences related to body size. The fact that oseltamivir is a high extraction drug may also explain why conversion of oseltamivir to OC is not appreciably altered in subjects with moderate hepatic impairment (Child-Pugh score <10) (7).

Given that the OC metabolite is predominantly renally cleared and that adult subjects with different degrees of renal function were included in the PK database, it was not surprising that both body weight and CLCR were statistically significant predictors of CL/F (power function exponents were 0.56 and 0.49, respectively). The ability to elucidate the impact of both body size and renal function on CL/F was made possible by normalizing the calculated CL/F in adults ≥18 years of age to a BSA of 1.73 m². This normalization of CL/F also facilitated the combination of the adult data with the pediatric data in which CLCR was calculated in units of ml/min/1.73 m² via the Schwartz equation (16). For a 70-kg adult subject with a CLCR of 95 ml/min/1.73 m², CL/F was estimated to be 20.7 liters/h and was comparable to previously reported values (10, 18). Since OC displays formation rate-limited kinetics due to the release rate of OC from hepatocytes (8, 9), CL/F in adult subjects with normal renal function likely does not reflect the actual renal clearance of OC. The same should hold true for pediatric subjects given that GFR normalizes to adult values by the first year of age (24, 25). However, in moderate and severe renal impairment, CL/F likely reflects the renal clearance of OC, as this is now the rate-limiting step (8, 20). The lack of data in subjects with CLCR of <30 ml/min/1.73 m² limits the ability to use the model to confirm dose adjustment recommendations for oseltamivir in severe renal dysfunction; however, the current recommendation is to reduce the oral oseltamivir dose by 50% for subjects with severe renal impairment (CLCR of 11 to 30). In the current analysis, a 0.51- to 0.71-fold decrease in CL/F was predicted for subjects with moderate renal dysfunction (CLCR of 30 to 60 ml/min/1.73 m² relative to a CLCR of 120 ml/min/1.73 m²),

FIG 5 Plot of the individual post hoc predicted (Bayesian) versus population mean predicted OC Cmax and AUC0–24 obtained using the final population PK model. The broken gray line represents a linear regression fit, while the solid black line represents the line of identity.
indicating that dosage adjustment may also be warranted for moderate renal impairment.

The apparent volume of distribution for OC (Vcm/F) was estimated to be 238 liters, and as expected, it also increased according to a power function with weight. The estimate of the power coefficient was 0.83, which was slightly lower than the theoretical value of 1 traditionally used in allometry. A slight decrease in Vcm/F with age was also detected, which may be indicative of age-related changes in body composition (26). Polar drugs that are mainly water soluble tend to have smaller volumes of distribution in the elderly, resulting in higher plasma drug concentrations. However, while OC exposures were previously reported to be increased by as high as 80% in the elderly, the drug was shown to be well tolerated by these subjects (27). Simulations performed using the population PK model (Fig. 4) also demonstrated that after accounting for body weight and renal function, age-related changes in OC exposures are not clinically significant.

In summary, the results of the analysis described herein represent the most comprehensive population PK analysis conducted thus far for orally administered oseltamivir and its active OC metabolite. Subject covariates were identified to help explain PK variability and to bridge the data collected from pediatric to adult subjects. There also appeared to be reasonable concordance between the population and individual predictions of OC Cmax and AUC0–24 for subjects included in this analysis, inferring that the population PK model can approximate OC exposure metrics in patients with only demographic data. The individual predicted OC exposure metrics (or population predicted OC exposures for subjects without measured PK data) from subjects in the two phase 2 influenza inoculation studies included in the pooled PK data set were subsequently used to explore PK-PD relationships with virologic and clinical efficacy endpoints in a companion paper by Rayner et al. (28).

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