A Prospective Intervention Study on Higher-Dose Oseltamivir Treatment in Adults Hospitalized With Influenza A and B Infections


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Background. It is unclear if higher-dose oseltamivir provides benefit beyond the standard dose in influenza patients who require hospitalization.

Methods. A prospective intervention study was performed in 2 acute care general hospitals in Hong Kong over 4 seasonal peaks (2010–2012). Adults (≥18 years) with laboratory-confirmed influenza (85 A/H3N2, 34 A/H1N1pdm09, 36 B) infections who presented within 96 hours were recruited. Study regimen of either 150 mg or 75 mg oseltamivir twice daily for 5 days was allocated by site, which was switched after 2 seasons. Subjects with preexisting renal impairment (creatinine clearance, 40–60 mL/minute) received 75 mg oseltamivir twice daily. Viral clearance by day 5 and clinical responses were compared between groups. Plasma steady-state trough oseltamivir carboxylate (OC) concentration was measured by high-performance liquid chromatography–tandem mass spectrometry.

Results. Altogether, 41 and 114 patients received 150 mg and 75 mg twice-daily oseltamivir, respectively; their enrollment characteristics (mean age, 61 ± 18 vs 66 ± 16 years) and illness severity were comparable. Trough OC levels were higher in the 150-mg group (501.0 ± 237.0 vs 342.6 ± 192.7 ng/mL). There were no significant differences in day 5 viral RNA (44.7% vs 40.2%) or culture negativity (100.0% vs 98.1%), RNA decline rate, and durations of fever, oxygen supplementation, and hospitalization. Results were similar when analyzed by study arm (all cases and among those without renal impairment). Subanalysis of influenza B patients showed faster RNA decline rate (analysis of variance, F = 4.14; P = .05) and clearance (day 5, 80.0% vs 57.1%) with higher-dose treatment. No oseltamivir resistance was found. Treatments were generally well tolerated.

Conclusions. We found no additional benefit of higher-dose oseltamivir treatment in adults hospitalized with influenza A, but an improved virologic response in influenza B.

Clinical Trials Registration. ClinicalTrials.gov, NCT01052961.

Keywords. influenza; high dose; oseltamivir; treatment; viral clearance.

Influenza can cause serious illness leading to hospitalization and death. Treatment with neuraminidase inhibitors may shorten illness duration and improve clinical outcomes and should be considered in patients with severe infections [1]. The standard regimen of oseltamivir, 75 mg twice daily for 5 days, however, was based on clinical trials in ambulatory patients with mild seasonal influenza [2, 3]. Because hospitalized adult patients have a different clinical course, it is questioned whether such a standard dose is adequate. Oseltamivir given at higher doses has been shown to be safe and well tolerated [2–6], and dosing regimens such as 150 mg twice daily have been used to treat seriously ill patients [7–9]. However, supportive evidence is lacking [7]. In this study, we aimed to investigate the use of higher-dose (150 mg

METHODS

Study Design and Case Recruitment

A prospective, open-label, intervention study was conducted during 4 influenza seasonal peaks from January 2010 to June 2012 (additional peak, “second wave” of A/H1N1pdm09 in 2010) [10] in 2 general acute-care hospitals in Hong Kong. Inclusion criteria were age ≥18 years, hospitalization for influenza A or B infection that was confirmed by immunofluorescence assay or polymerase chain reaction (PCR), presentation within 96 hours from illness onset, and provision of written informed consent. Exclusion criteria included receipt of any antiviral for influenza before presentation, preexisting severe renal impairment (creatinine clearance [CrCl] <40 mL/minute), hepatic failure, pregnancy, and lactation.

There were 2 study arms: (1) standard therapy, oseltamivir 75 mg twice daily for 5 days, given based on existing recommendations [1, 7], and (2) the active comparator, oseltamivir 150 mg twice daily for 5 days. Patients with moderate renal impairment (defined as CrCl 40–60 mL/minute) in this arm received an adjusted dosage of 75 mg twice daily, as available data suggest substantial increase in drug exposure because of slow drug elimination [4, 5, 11, 12]. Study arms were allocated by site (see Discussion); each hospital was first randomly assigned to one arm then switched to the other after 2 seasonal peaks had elapsed, so that both hospitals had patients recruited to receive the active comparator.

Hospital admission procedures and management of our influenza patients have been described previously [13, 14]. In brief, patients presenting with acute respiratory illnesses were hospitalized if they had developed potentially serious complications, exacerbation of underlying illnesses, or severe constitutional and respiratory symptoms. Nasopharyngeal aspirates (or swabs in some cases) were collected for virus testing, regardless of perceived etiology and severity [13, 14]. Sputum bacterial culture was routinely performed; blood culture was performed when there were signs of sepsis [13–16]. Once diagnosed, influenza patients received cohort isolation at designated ward cubicles for strict droplet precautions, and were assessed for study eligibility [15, 16]. Ethical approval was obtained from the institutional review boards of the Chinese University of Hong Kong and the Hospital Authority of Hong Kong.

Sampling and Data Collection

Nasopharyngeal flocked swabs (NPFSs) were collected at time of recruitment (baseline = day 0) and then daily to assess virologic response until completion of the treatment course [15, 16]. Patients discharged early had follow-up visits for assessment and sample collection. In a subset of consecutively recruited patients, 1 blood sample was collected after the fourth dose, just before taking the next dose (ie, at trough; approximately 11–13 hours postdose) [4, 5], to estimate the steady-state plasma concentration of oseltamivir and its active carboxylate metabolite (see Table 1 footnotes). Clinical data were prospectively collected using a standardized research tool, which included demographics, comorbidities, vaccination status, complications, symptom scores, vital signs, concomitant medications, and outcome measures including duration of hospitalization, fever resolution, and oxygen supplementation; intensive care unit (ICU) admission; and death [13–16]. Baseline CrCl was calculated for each patient using the Cockroft-Gault equation, as described elsewhere [17].

Virologic Investigations

All nasopharyngeal samples collected at presentation for diagnosis were subjected to influenza A, influenza B, respiratory syncytial virus, parainfluenza viruses 1–3, and adenovirus detection using immunofluorescence or PCR assays, and concomitant influenza virus culture [14–16].

The serially collected NPFSs for study were subjected to both viral RNA (viral load) quantification and virus isolation. Influenza A and B viral RNA was measured by quantitative reverse transcription PCR targeting the M-gene (SuperScript III Platinum One-Step qRT-PCR Kit w/ROX; Invitrogen, Carlsbad, California), using methods previously described [15, 16, 18]. The lower detection limits for influenza A and B virus RNA were 250 copies/mL and 200 copies/mL, respectively. Virus isolation was performed using Madin-Darby canine kidney cells [14, 15].

To detect possible oseltamivir resistance, samples that remained culture positive after 5 days of treatment were tested by the NA-Star Influenza Neuraminidase Inhibition Resistance Detection Assay (Applied Biosystems). Samples that remained PCR positive beyond 5 days were analyzed by sequencing covering the gene regions encoding for the catalytic sites and framework sites of neuraminidase. Mutations known to be associated with resistance to neuraminidase inhibitors are located within these regions (eg, E119 V, I222 V, H274Y, R292 K, N294S; N2-numbering) [19].

Plasma Oseltamivir Carboxylate and Oseltamivir Phosphate Assays

Blood samples were collected using sodium fluoride and potassium oxalate tubes to prevent in vitro hydrolysis of oseltamivir carboxylate (OP) [20, 21]. Plasma concentrations of oseltamivir phosphate (OP) and OC (free base) were analyzed using high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS), with modifications [22]. In brief,
### Table 1. Baseline Characteristics, Illness Severity, and Plasma Drug Concentrations

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Active Comparator Arm (n = 70)</th>
<th>Standard Therapy Arm (n = 87)</th>
<th>P Value</th>
<th>150 mg bid Recipients (n = 41)</th>
<th>75 mg bid Recipients (n = 114)</th>
<th>Subgroup 75 mg bid Recipients, CrCl &gt;60 mL/min</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Influenza A, %</td>
<td>72.9</td>
<td>78.2</td>
<td>.441</td>
<td>75.6</td>
<td>77.2</td>
<td>83.7</td>
<td>.857</td>
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<tr>
<td>Influenza B, %</td>
<td>27.1</td>
<td>21.8</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Age, y, mean ± SD</td>
<td>66.8 ± 16.5</td>
<td>62.7 ± 17.4</td>
<td>.137</td>
<td>61.3 ± 18.1</td>
<td>66.2 ± 16.1</td>
<td>55.6 ± 17.1</td>
<td>.129</td>
</tr>
<tr>
<td>Sex, male, %</td>
<td>61.4</td>
<td>63.2</td>
<td>.818</td>
<td>61.0</td>
<td>63.2</td>
<td>.804</td>
<td>.673</td>
</tr>
<tr>
<td>Comorbidity, systemic, %</td>
<td>55.7</td>
<td>42.5</td>
<td>.100</td>
<td>43.9</td>
<td>50.0</td>
<td>.503</td>
<td>.347</td>
</tr>
<tr>
<td>Chronic lung disease, %</td>
<td>37.1</td>
<td>29.9</td>
<td>.337</td>
<td>39.0</td>
<td>31.6</td>
<td>.387</td>
<td>.286</td>
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<tr>
<td>Vaccination history, %</td>
<td>16.1</td>
<td>20.7</td>
<td>.430</td>
<td>14.3</td>
<td>20.6</td>
<td>.412</td>
<td>.618</td>
</tr>
<tr>
<td>Baseline viral load, log₁₀ copies/mL, mean ± SD</td>
<td>8.5 ± 1.5</td>
<td>8.9 ± 1.6</td>
<td>.212</td>
<td>8.5 ± 1.5</td>
<td>8.8 ± 1.6</td>
<td>.209</td>
<td>8.9 ± 1.6</td>
</tr>
<tr>
<td>Baseline total symptom score, mean ± SD</td>
<td>7.1 ± 4.7</td>
<td>7.9 ± 4.5</td>
<td>.346</td>
<td>8.3 ± 5.3</td>
<td>7.2 ± 4.3</td>
<td>.219</td>
<td>7.5 ± 4.4</td>
</tr>
<tr>
<td>Cardiorespiratory complications, %</td>
<td>74.3</td>
<td>58.6</td>
<td>.040</td>
<td>75.6</td>
<td>63.2</td>
<td>.148</td>
<td>.612</td>
</tr>
<tr>
<td>Use of supplemental oxygen, %</td>
<td>45.7</td>
<td>43.7</td>
<td>.799</td>
<td>46.3</td>
<td>44.7</td>
<td>.859</td>
<td>.347</td>
</tr>
<tr>
<td>Secondary bacterial infection, %</td>
<td>11.4</td>
<td>8.0</td>
<td>.474</td>
<td>9.8</td>
<td>9.6</td>
<td>.984</td>
<td>.261</td>
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<tr>
<td>Antibacterial treatment, %</td>
<td>95.7</td>
<td>94.3</td>
<td>.679</td>
<td>97.6</td>
<td>93.9</td>
<td>.358</td>
<td>.623</td>
</tr>
<tr>
<td>Time from onset to oseltamivir initiation, d, IQR (100–200)</td>
<td>2.0 (1.0–2.0)</td>
<td>2.0 (1.0–2.0)</td>
<td>.595</td>
<td>2.0 (1.0–2.0)</td>
<td>2.0 (1.0–2.0)</td>
<td>.668</td>
<td>2.0 (1.0–3.0)</td>
</tr>
<tr>
<td>Steady-state OC concentration, ng/mL, mean ± SD</td>
<td>626.3 ± 314.4439.1 ± 248.6 ± .005</td>
<td>501.0 ± 237.0 ± 192.7. .004</td>
<td>342.6 ± 119.4 ± .265</td>
<td>264.5 ± 114.9 ± .001</td>
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<tr>
<td>Steady-state OP concentration, ng/mL, mean ± SD</td>
<td>7.1 ± 20.2 ± 2.9 ± 9.6 ± 250</td>
<td>7.9 ± 19.6 ± 2.1 ± 6.3 ± .054</td>
<td>2.6 ± 9.4 ± .242</td>
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</table>

**Abbreviations:** bid, twice daily; CrCl, creatinine clearance; IQR, interquartile range; OC, oseltamivir carboxylate; OP, oseltamivir phosphate; SD, standard deviation.

*Subgroup comparisons between 75 mg twice-daily recipients in the standard therapy arm and without significant renal impairment (ie, CrCl >60 mL/minute), and those who received 150 mg twice-daily oseltamivir in the active comparator arm. Proportion of patients with baseline CrCl >60 mL/minute in the active-comparator standard therapy arm was 59% and 56%, respectively. Oseltamivir >5 days was given to 3 and 2 patients in the 75 mg and 150 mg twice-daily regimen groups, respectively.

*Virologic diagnosis: positive immunofluorescence assay, 147/155; positive virus culture, 128/155; all 155 treated cases were confirmed by polymerase chain reaction with viral load measured. Proportions of A/H3N2 in the active comparator vs standard therapy arm, 56% vs 53%, respectively. Oseltamivir-resistant seasonal A/H1N1 virus was not in circulation during the study period [10].

*Comorbidity, systemic: Major systemic comorbidity—congestive heart failure, cerebrovascular, neoplastic, chronic liver and renal diseases; other chronic cardiovascular and neurological conditions (except hypertension), diabetes mellitus, autoimmune disorders, and use of immunosuppressants. Chronic lung diseases: chronic obstructive pulmonary disease, asthma, bronchiectasis, and pulmonary fibrosis. Patients may have >1 comorbid illness. Vaccination status: history of influenza vaccination prior to that season (unknown status, n = 15) [13–16].

*Cardiorespiratory complications: lower respiratory tract complications—pneumonia, bronchitis, exacerbations of chronic pulmonary diseases (n = 101), and/or acute cardiovascular and cerebrovascular events—congestive heart failure, arrhythmia, stroke, etc. Patients might have >1 complication. Symptom score: respiratory and systemic symptoms scored on a 4-point scale as previously described [15]. Secondary bacterial infection: evidenced by positive culture of a bacterial pathogen from respiratory and/or blood samples, other than commensals such as oral flora [13–16].

*Steady-state OC/OP concentration: plasma OC and OP concentrations were measured in 75 consecutive patients (150 mg twice daily, n = 21; 75 mg twice daily, n = 54) after a median of 5 (IQR, 5–7) doses of oseltamivir had been received, and before taking the next dose (ie, trough concentration, at a median of 11 [IQR, 11–13] hours postdose) [4–6].

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200 µL of plasma with addition of internal standard (cephalexin hydrate) was extracted with an Oasis MCX cartridge. The analytes were separated by a Nova-Pak CN HP column (75 x 3.9 mm internal diameter, 4 µm, Waters) using 0.1% formic acid:methanol (1:1 v/v) at 1 mL/minute, and detected in multiple reaction monitoring mode (electrospray positive ionization) with m/z 313 → m/z 166 for OP, m/z 285 → m/z 138 for OC and m/z 348 → m/z 158 for IS. The linearity of analytes was 2–1000 ng/mL for OP and 10–10 000 ng/mL for OC. The assay was validated according to the Guidance for Industry Bioanalytical Method Validation from the US Food and Drug Administration [23].

**Statistical Analysis**

The Student t test, Mann-Whitney U test, and χ² or Fisher exact tests were used for univariate comparisons whenever appropriate. Spearman rank correlation coefficient was used to examine correlation between plasma OC concentration and renal function (CrCl). Rates of PCR negativity (ie, below detection limits) [15] and culture negativity at 5 days after starting...
treatment were compared between the (1) active comparator and standard therapy arms (intention-to-treat), and (2) recipients of oseltamivir 150 mg and 75 mg twice daily (ie, actual received doses). Patients in each arm with CrCl >60 mL/minute (and thus received the full intended doses) were also compared. Independent factors associated with lack of viral RNA clearance (ie, PCR positivity) at day 5 were examined using backward, stepwise logistic regression models (conditional; probability of entry <.05, removal >.1); adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each explanatory variables. Longitudinal changes in viral RNA concentration during the treatment course was analyzed using repeated-measures analysis of variance (ANOVA) to examine for interactions between serial RNA concentrations and clinical and treatment variables [15]. Clinical outcomes between the treatment groups were also compared. Multivariate Cox proportional hazards model analyses were performed to determine independent factors associated with hospital discharge, duration of oxygen supplementation, or time to fever resolution [13, 14]. In all analyses, 2-tailed P values <.05 were regarded as statistically significant. Analyses were performed using PASW Statistics software, version 17.0 (SPSS Inc, Chicago, Illinois).

RESULTS

Descriptions of Study Patients, Treatment, and Plasma Drug Levels

Altogether, 157 patients were recruited, with 87 patients in the standard therapy arm and 70 patients in the active comparator arm. Eighty-five patients in the standard therapy arm received oseltamivir at 75 mg twice daily; 2 patients with mild illness received no antiviral. In the active comparator arm, 41 (59%) received oseltamivir at 150 mg twice daily, and 29 (41%) received no antiviral. In the active comparator arm. Eighty-six patients in the standard therapy arm and 70 patients in the active comparator arm. Eighty-five patients in the standard therapy arm received oseltamivir at 75 mg twice daily; 2 patients with mild illness received no antiviral. In the active comparator arm, 41 (59%) received oseltamivir at 150 mg twice daily, and 29 (41%) received no antiviral. In the active comparator arm (Table 1). The steady-state, trough plasma OC concentrations were shown to have significant, negative correlations with patients’ renal function (Spearman ρ = −0.325; P = .004; Figure 1).

Comparisons of Virologic Outcomes

A median of 6 (interquartile range [IQR], 5–7) NPFSs were collected from each patient from day 0 to day 5 after starting oseltamivir treatment for assessment. Overall, 58.5% (n = 76) of cases showed persistently positive PCR results at day 5, whereas 8.4% (n = 12) showed positive culture results at day 3, and only 1.3% (n = 2) at day 5. Relationships between viral load and culture positivity are described in Supplementary Data S1. Patients with positive PCR results at day 5 had longer durations of hospitalization (median, 6.0 [IQR, 4.0–9.0] vs 4.0 [IQR, 3.0–6.3] days, P = .004), time to fever resolution (2.0 [IQR, 1.0–3.0] vs 1.0 [IQR, 0.0–2.0] days, P = .126), and supplemental oxygen therapy (4.0 [IQR, 2.0–6.3] vs 2.0 [IQR, 1.0–3.0] days, P = .059). We did not detect phenotypic oseltamivir resistance in the 2 isolates (1 A/H3N2, 1 B) obtained at day 5. Among samples showing persistent RNA positivity beyond day 5, only 7 (6 A/H3N2, 1 A/H1N1pdm09) had high enough concentrations to allow sequencing analysis. No mutations that had been reported to
associate with oseltamivir resistance were found by neuraminidase sequencing.

We found no significant difference in viral RNA negativity at day 5 between recipients of the 150-mg and 75-mg oseltamivir regimens (44.7% vs 40.2%); results were similar if analyzed by study arm (all cases, 39.7% vs 43.3%; without renal impairment, 44.7% vs 47.5%; Table 2). Notably, there was a trend toward more frequent day 5 RNA negativity with 150-mg treatment in influenza B patients (80.0% vs 57.1%; P = .214), but not in those with influenza A (32.1% vs 35.2%). Comparisons on culture negativity at day 3 and day 5 showed no significant differences between treatment groups. The mean steady-state OC concentration did not significantly differ between cases with or without day 5 viral RNA clearance (543.0 ± 290.6 vs 505.2 ± 306.0 ng/mL). Further comparisons on viral clearance in relation to renal function, drug level, and dosing regimens are provided in Supplementary Data S2.

Multivariate logistic regression analyses showed that lack of viral RNA clearance at day 5 was associated with higher baseline virus concentration, clinical characteristics, and cardiorespiratory complications, but not with dosing regimen (Table 3). However, subgroup analysis of influenza B cases showed a trend toward faster viral RNA clearance with 150 mg twice-daily treatment (adjusted OR, 0.17 [95% CI, .02–1.36]; P = .094), adjusted for baseline virus concentration, clinical characteristics, and cardiorespiratory complications.

Analyses of the longitudinal viral load changes showed that for influenza A cases, only presence of cardiorespiratory complications (ANOVA: F = 9.197, P = .003) but not the oseltamivir dose received (150 mg vs 75 mg, P = .826) was associated with slower viral RNA decline, adjusted for confounders (Figure 2A). Subanalyses of patients without significant renal impairment (150 mg vs 75 mg, P = .967), and separately for A/H3N2 or A/H1N1pdm09 influenza showed similar results. For influenza B, however, we found a trend toward significance with the 150-mg regimen being associated with faster rate of viral RNA decline, adjusted for confounders (ANOVA: F = 4.141, P = .051; Figure 2B).

**Comparisons of Clinical Outcomes**

We found no significant difference in hospitalization duration, time to discontinuation of supplemental oxygen, or time to fever resolution between treatment groups in univariate analyses (Table 2). Few patients developed critical illness; no 150-mg oseltamivir regimen recipient required ICU admission, vs 2 among the 75-mg regimen recipients. No patient died during the course of treatment; 1 patient in each group who had completed treatment died of advanced chronic obstructive pulmonary disease later after a long period of hospitalization. Multivariate Cox regression analyses, adjusted for potential confounders, showed no significant difference in durations of hospitalization, oxygen supplementation, and fever between the treatment groups (Supplementary Data S3). Advanced age, cardiorespiratory

**Table 2. Virologic and Clinical Outcomes**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Active Comparator Arm (n = 70)</th>
<th>Standard Therapy Arm (n = 87)</th>
<th>150 mg bid Recipients (n = 41)</th>
<th>75 mg bid Recipients (n = 114)</th>
<th>Subgroup 75 mg bid Recipients, CrCl &gt;60 mL/min&lt;sup&gt;a&lt;/sup&gt; (n = 49)</th>
<th>P Value&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Virologic</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>PCR negativity at day 5, %</td>
<td>39.7</td>
<td>43.3</td>
<td>.677</td>
<td>44.7</td>
<td>40.2</td>
<td>.634</td>
</tr>
<tr>
<td>Culture negativity at day 3, %</td>
<td>98.2</td>
<td>94.7</td>
<td>.229</td>
<td>90.0</td>
<td>92.2</td>
<td>.739</td>
</tr>
<tr>
<td>Culture negativity at day 5, %</td>
<td>98.6</td>
<td>98.7</td>
<td>&gt;.99</td>
<td>100.0</td>
<td>98.1</td>
<td>&gt;.99</td>
</tr>
<tr>
<td><strong>Clinical</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Duration of hospitalization, d, median (IQR)</td>
<td>6.0 (3.0–8.0)</td>
<td>4.0 (3.0–6.5)</td>
<td>.138</td>
<td>5.0 (3.0–7.8)</td>
<td>5.0 (3.0–7.0)</td>
<td>.943</td>
</tr>
<tr>
<td>Duration of oxygen therapy, d, median (IQR)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0 (1.3–5.8)</td>
<td>3.0 (1.0–5.0)</td>
<td>.704</td>
<td>3.0 (1.0–6.5)</td>
<td>3.0 (1.0–5.0)</td>
<td>.789</td>
</tr>
<tr>
<td>Duration of fever &gt;37.5°C, d, median (IQR)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.5 (0.0–3.0)</td>
<td>1.0 (1.0–2.0)</td>
<td>.982</td>
<td>2.0 (0.0–3.0)</td>
<td>1.0 (1.0–2.0)</td>
<td>.482</td>
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<tr>
<td>ICU admission, %</td>
<td>0.0</td>
<td>2.3</td>
<td>&gt;.99</td>
<td>0.0</td>
<td>1.8</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Death, %</td>
<td>1.4</td>
<td>1.1</td>
<td>&gt;.99</td>
<td>2.4</td>
<td>0.9</td>
<td>&gt;.99</td>
</tr>
<tr>
<td>ICU admission or death, %</td>
<td>1.4</td>
<td>3.4</td>
<td>&gt;.99</td>
<td>2.4</td>
<td>2.6</td>
<td>&gt;.99</td>
</tr>
</tbody>
</table>

Abbreviations: bid, twice daily; CrCl, creatinine clearance; ICU, intensive care unit; IQR, interquartile range; PCR, polymerase chain reaction.

<sup>a</sup> Subgroup comparisons between 75 mg twice-daily recipients in the standard therapy arm and without significant renal impairment (ie, CrCl >60 mL/minute) and those who received 150 mg twice-daily oseltamivir.

<sup>b</sup> Virologic outcomes were evaluated by PCR and culture at day 3 and day 5 after starting oseltamivir treatment (±0.5 day). Three and 22 patients in the 150 mg and 75 mg twice-daily regimen groups, respectively, had virologic data up to day 3 due to early recovery and discharge. Clinical outcome data were available for all study patients.

<sup>c</sup> Duration between start of supplemental oxygen therapy and its termination, or resumption of baseline oxygen requirement in patients who required long-term oxygen therapy. Duration of fever after starting on oseltamivir (not day from onset).
complications, and lack of viral RNA clearance were factors independently associated with longer hospitalization durations.

**Tolerance to Oseltamivir Treatment**

Two patients on the 75-mg oseltamivir regimen and 1 patient on the 150-mg regimen discontinued treatment after 1–3 days because of suspected drug intolerance (1.8% vs 2.4%; *P* > .99); 3 other patients initially on 150 mg twice daily had subsequent dosage reduction to 75 mg twice daily and completed the treatment course (Supplementary S4). Four of these 6 patients were receiving concomitant antibiotics. Per-protocol analyses excluding these few cases did not change the results on virologic outcomes (data not shown).

**DISCUSSION**

We found no significant differences in virologic and clinical outcomes between 150 mg and 75 mg twice-daily oseltamivir dosing regimen (150 mg vs 75 mg twice daily) was included as a covariate. Other covariates in the model included comorbidities, baseline creatinine clearance values, and corticosteroid use for chronic obstructive pulmonary disease/asthma. Day 5 polymerase chain reaction positivity among corticosteroid treated and untreated patients was 62.9% and 55.4%, respectively [13]. Influenza A/H1N1pdm09 was shown to associate with higher chance of day 5 RNA positivity in the model when compared with influenza A/H3N2 or B [16]. Elderly patients (>65 years) had been shown to have more prolonged viral shedding and poorer outcomes [1, 13, 15]. Analysis using study arm as a covariate (active comparator vs standard therapy) showed similar results. Abbreviations: CI, confidence interval; OR, odds ratio.

### Table 3. Factors Associated With Lack of Viral RNA Clearance at 5 Days After Starting Oseltamivir, as Shown in the Final Logistic Regression Model

<table>
<thead>
<tr>
<th>Explanatory Variables</th>
<th>Adjusted OR (95% CI)</th>
<th><em>P</em> Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline nasopharyngeal viral RNA concentration, log10 copies/mL</td>
<td>1.79 (1.22–2.62) (per 1 log increase)</td>
<td>.003</td>
</tr>
<tr>
<td>Age &gt;65 y</td>
<td>3.04 (1.22–7.64)</td>
<td>.018</td>
</tr>
<tr>
<td>Sex, female</td>
<td>0.29 (0.12–0.71)</td>
<td>.007</td>
</tr>
<tr>
<td>Presence of cardiorespiratory complications</td>
<td>2.39 (1.00–5.71)</td>
<td>.051</td>
</tr>
<tr>
<td>Influenza A/H3N2</td>
<td>0.23 (0.07–0.79)</td>
<td>.019</td>
</tr>
<tr>
<td>Influenza B</td>
<td>0.33 (0.09–1.27)</td>
<td>.107</td>
</tr>
</tbody>
</table>

Oseltamivir dosing regimen (150 mg vs 75 mg twice daily) was included as a covariate. Other covariates in the model included comorbidities, baseline creatinine clearance values, and corticosteroid use for chronic obstructive pulmonary disease/asthma. Day 5 polymerase chain reaction positivity among corticosteroid treated and untreated patients was 62.9% and 55.4%, respectively [13]. Influenza A/H1N1pdm09 was shown to associate with higher chance of day 5 RNA positivity in the model when compared with influenza A/H3N2 or B [16]. Elderly patients (>65 years) had been shown to have more prolonged viral shedding and poorer outcomes [1, 13, 15]. Analysis using study arm as a covariate (active comparator vs standard therapy) showed similar results. Abbreviations: CI, confidence interval; OR, odds ratio.

In half of these cases (n = 7), the symptoms resolved after stopping or modifying the concomitant medications without discontinuing oseltamivir. All events were mild to moderate and reversible.

**Figure 2.** Longitudinal viral RNA concentration changes after starting oseltamivir treatment, shown according to dosing regimens and influenza virus subtype. A, Influenza A. Repeated-measures analysis of variance (ANOVA); covariates included oseltamivir regimen (150 mg vs 75 mg twice daily; *F* = 0.049, *P* = .826), cardiorespiratory complications (*F* = 9.197, *P* = .003), age, sex, comorbidity, and corticosteroid use. Analysis comparing 150 mg vs 75 mg twice-daily recipients in the standard therapy group showed similar results (*P* = .967). The lower detection limit of the assay was 2.4 log10 (ie, 250) copies/mL. B, Influenza B. Repeated-measures ANOVA; covariates included oseltamivir regimen (150 mg vs 75 mg twice daily; *F* = 4.141, *P* = .051); presence of cardiorespiratory complications, age, sex, comorbidity, and corticosteroid use. Further comparison between 150 mg vs 75 mg twice-daily recipients in the standard therapy group showed similar results (*P* = .097). The lower detection limit of the assay was 2.3 log10 (ie, 200) copies/mL. Abbreviation: bid, twice daily.
treatment regimens in adults hospitalized with influenza A. For influenza B, a trend toward faster viral clearance was observed with the higher-dose treatment. Our results are consistent with another trial performed in Southeast Asia that involved mainly pediatric patients hospitalized for severe seasonal or (uncommonly) avian A/H5N1 influenza. Double-dose oseltamivir, while safe, did not seem to offer additional benefit in shortening clinical illness or duration of viral shedding [25]. Data from several observational studies on severe avian A/H5N1 and pandemic A/H1N1pdm09 infections also showed no additional benefit with higher-dose regimens [1–3, 16, 26, 27]. Oseltamivir is generally well absorbed (even in the critically ill) [1, 4, 5, 27], and rapidly hydrolyzed by liver carboxylesterase to produce the active compound, oseltamivir carboxylate, with a bioavailability exceeding 80% [4, 5]. In young adults with normal renal function, the average steady-state maximum and minimum (trough) plasma OC concentrations achieved with the 75-mg twice-daily regimen are approximately 300–400 ng/mL and approximately 150–250 ng/mL, respectively (our assay has shown comparable results for this group) (Figure 1) [4, 5, 24]. Although the precise pharmacokinetic-pharmacodynamic (PK-PD) relationships for oseltamivir are unclear, these OC levels already greatly exceed the in vitro inhibitory concentrations of most circulating influenza A viruses (>1000-fold of 50% inhibitory concentration [IC50] values of A/H3N2 approximately 0.07–0.11 ng/mL, and A/H1N1pdm09 approximately 0.09–0.19 ng/mL; 90% inhibitory concentration [IC90] values are approximately 8–18 times higher, depending on assay methods), so that an antiviral effect can be expected [1, 27–30].

In clinical practice, patients hospitalized for seasonal influenza are mostly of older age with underlying medical conditions; renal clearance of OC in such patients can be reduced, resulting in higher drug exposure [4, 5, 11, 12]. We observed high trough OC levels in these patients treated with 75 mg twice-daily oseltamivir (Figure 1); in some cases, the values resembled those reported in critically ill A/H5N1 or A/H1N1pdm09 influenza patients [1, 27]. Because of the high safety margin of oseltamivir, dosage reduction is only recommended in those with CrCl <30 mL/minute [4–6]. Our analyses showed that increasing oseltamivir dosage in these hospitalized, older adults was not associated with better viral clearance or clinical response. Comparatively, the inhibitory concentrations of OC for influenza B are much higher (IC50, approximately 1.4–2.4 ng/mL; IC90, approximately 16–18 times higher); the relative plasma levels attained with standard dosing may be in the range of 10–100-fold [27–30, 31]. Suboptimal virologic and clinical responses of influenza B have been reported in both children and adults receiving standard therapy [15, 30, 32]. Notably, we found improved clearance of this less susceptible virus with higher-dose oseltamivir, albeit with a smaller sample size. Given the burden and severity of influenza B [33], further study on oseltamivir treatment dosage to achieve maximal viral suppression and clinical benefit is warranted.

Our study also delineated the virologic response to oseltamivir treatment in hospitalized adults. By day 5 of treatment, 98.7% of patients had shown negative culture results, and there was no evidence of emergence of oseltamivir resistance; viral RNA load was reduced by about 1000-fold (3 logs) from baseline (Figure 2) [15, 34]. Of note, in about half of the cases, viral RNA remained detectable by the end of the intended treatment course (affected by host, virologic, and other treatment-related factors, such as viral level at time of antiviral initiation; Table 3) [15, 16, 25]. Although some positive PCR results likely represent nonviable viruses, the finding should not be neglected, because culture is known to have a much lower sensitivity [7, 35]. In fact, we are able to show significant correlations between persistent RNA detection and longer illness duration (eg, length of stay), even after adjustment for confounders [7, 15]. These data support the view that a standard 5-day treatment course may be inadequate in patients suffering from severe or complicated influenza [7, 8, 16]. Studies on treatment duration, coupled with the use of both culture and quantitative PCR to assess antiviral response, are urgently needed in this unique population [34].

Our study is first of all limited by the lack of individual randomization, as restricted by our clinical setting (eg, patients were cohorted in ward cubicles; no placebo capsule for double-dose was available). Therefore, potential confounders were carefully adjusted in multivariate analyses. Second, the sample size may be relatively small to detect minor differences in the virological or clinical outcomes. Because our study population had very few patients with critical illness, our conclusions may not apply to such patient groups, or those infected with avian influenza (A/H5N1, A/H7N9). Complete pharmacokinetic profiling and lower-respiratory tract sample collection are ideal but unrealistic in non-ICU settings. Even so, our data on trough concentrations reflected drug exposure associated with the higher or standard dose regimens in these older hospitalized adults. Because oseltamivir is generally well tolerated even at increased dosages (mainly GI side effects) [1, 4–6, 25, 26, 36], further study on its therapeutic window and the PK-PD relationship in selected influenza virus infections (eg, influenza B; some influenza A strains with reduced susceptibility) [27–30, 37, 38] and infection sites with limited drug penetration (eg, central nervous system, as in some H5N1 cases) [4–6, 39, 40] remains important. A loading-dose strategy to shorten the time to reach target steady-state drug level in cases of rapidly progressive influenza pneumonia also deserves study [36].

In conclusion, we found no additional benefit of higher-dose oseltamivir over the standard-dose regimen in non–critically ill adults hospitalized with influenza A infection. Our observation of improved viral clearance with higher-dose treatment in influenza B warrants further investigation.
Supplementary Data

Supplementary materials are available at Clinical Infectious Diseases online (http://cid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


