Comparison of single-dose intravenous peramivir with oral oseltamivir in patients with seasonal influenza virus infection: A phase III randomized, double-blind study

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Abstract

Antiviral medications with activity against influenza viruses are important in controlling influenza. We compared intravenous peramivir, a potent neuraminidase inhibitor, with oseltamivir in patients with seasonal influenza virus infection. In a multinational, multicenter, double-blind, double-dummy, randomized controlled study, patients aged ≥20 years with influenza A or B virus infection were randomly assigned to receive either a single intravenous infusion of peramivir (300 or 600 mg) or oral administration of oseltamivir (75 mg b.i.d. for 5 days). To demonstrate the non-inferiority of peramivir in reducing the time to alleviate influenza symptoms with hazard model analysis and a non-inferiority margin of 0.170, we planned to recruit 1,050 patients in Korea, Japan, and Taiwan. A total of 1,091 patients (peramivir: 300 mg: 364, 600 mg: 362; oseltamivir: 365) were included in the Intent-To-Treat Infected population. The median duration of influenza symptoms was 78.0, 81.0, and 81.8 h in the 300-mg, 600-mg, and oseltamivir groups, respectively. The hazard ratios of the 300- and 600-mg groups compared to the oseltamivir group were 0.946 (97.5% confidence interval (CI): 0.793, 1.129) and 0.970 (0.814, 1.157), respectively. Both peramivir groups were non-inferior to the oseltamivir group (97.5% CI <1.170). The overall incidence of adverse drug reactions was significantly lower in the 300-mg group, but the incidence of severe reactions was not different in either peramivir group compared to the oseltamivir group. Thus, a single intravenous dose of peramivir may be an alternative to a 5-day oral dose of oseltamivir for patients with seasonal influenza virus infection.

INTRODUCTION

Influenza epidemics occur during the winter months in temperate climates. Data from epidemiologic studies during the 2009 influenza A (H1N1) pandemic indicated that antiviral agents, especially neuraminidase inhibitors (NAIs) such as oseltamivir and zanamivir, are important for treating patients with influenza (8, 24, 25, 26).

However, these drugs are associated with some unresolved problems. In particular, oral or inhaled administration may be unfeasible in patients with a bad healthy condition (14, 21); their efficacy of these drugs has not been fully established in severe cases, and development of NAI-resistant viruses is increasingly recognized, particularly for A/H1N1 viruses resistant to oseltamivir (4, 6, 15). Consequently, new drugs are needed (7).

Peramivir is a NAI that inhibits influenza virus proliferation (1, 2). In a previous controlled, double-blind study, peramivir was found to significantly reduce the duration of influenza symptoms without safety concerns after single administration at doses of 300 and 600 mg as compared to placebo (12). In a parallel trial, we investigated the efficacy and safety of peramivir administered over multiple days at 300 mg or 600 mg/day in patients with high-risk factors for severe disease (13).

Because a single intravenous dose can improve compliance and reliably provide stable pharmacokinetics regardless of the patient's condition, peramivir promises to be an important anti-influenza agent if it shows comparable efficacy as the standard anti-influenza treatment. Oseltamivir, the leading anti-influenza agent, has mostly been evaluated in otherwise healthy adults with uncomplicated influenza whose treatment was initiated within 48 h of symptom onset. We therefore compared a single intravenous dose of peramivir with multiple doses of oseltamivir in patients aged 20 years or older with influenza A or B virus infection.

During the 2008-2009 season, most A/H1N1 viruses (Russian strain) carried the H274Y neuraminidase (NA) mutation, resulting in decreased susceptibility to oseltamivir. Therefore,

we also evaluated the efficacy of peramivir against oseltamivir-resistant viruses.

MATERIALS AND METHODS

Study design

Our study was a multicenter, double-blind, randomized, controlled study with dynamic allocation using the minimization method, and was conducted in 146 medical institutions in Japan, Korea, and Taiwan from November 2008 to April 2009. This period was before the emergence of the 2009 pandemic A/H1N1 influenza. The study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines.

Patients

Patients aged 20 years or older with influenza A or B virus infection who met the following inclusion criteria were enrolled: available for treatment within 48 hours of onset of influenza symptoms, fever with an axillary temperature of ≥38.0°C, at least two moderate to severe symptoms among seven symptoms (headache, muscle or joint pain, feverishness or chills, fatigue, cough, sore throat, and nasal stuffiness) due to influenza, and rapid antigen test (RAT) positive for influenza. The onset of influenza symptoms was defined as the time of the first increase of ≥1°C from the patient's normal body temperature or the occurrence of at least one of the seven symptoms listed above. The RAT kits used in the study were Rapid Testa FLU II and FLU stick (Sekisui Medical), Espline Influenza A&B (Fuji Rebio), and Capilia FLU A+B (Tauns). Exclusion criteria were impaired respiratory function, a history of congestive cardiac failure, poorly controlled diabetes mellitus, immunosuppressive therapy (immunosuppressants, antitumor agents, etc.) or an immunodeficiency disorder such as acquired immunodeficiency syndrome (AIDS), renal disorder (estimated creatinine clearance <50 mL/min), ischemic heart disease or serious arrhythmia, a QTc of ≥480 msec or bradycardia (heart rate <40 bpm), clinically significant disorders that required hospitalization, and infection requiring systemic antimicrobial treatment.

Prior to enrolling each patient in the study, the investigator or subinvestigator provided him/her with written patient information (reviewed and approved by the IRB at each institution) and gave a detailed explanation to obtain voluntary written informed consent.

Procedures

Using a minimization method, patients were randomly assigned in a 1:1:1 ratio to receive peramivir at a dose of 300 or 600 mg (Shionogi, Osaka, Japan) or oseltamivir stratified on the basis of the composite symptom score (≤14/≥15), current smoking behavior (yes/no), country, and influenza virus type revealed by RAT for the diagnosis of influenza. Peramivir was administered as a single intravenous infusion of 60 to 100 mL over 15 to 60 min. Oseltamivir was administered orally at a dose of 75 mg twice daily for 5 days. Blinding was maintained by the double-dummy technique using two placebos identical to peramivir and oseltamivir. The concomitant use of the antipyretic acetaminophen was allowed, but other antipyretics, antivirals and antimicrobials were not permitted.

All patients returned to the investigational site for protocol-required assessments at days 1 (baseline), 2 (optional), 3, 8, and 14. Laboratory tests were performed on day 1 (baseline), 3, and 8 and included hematological examination (white blood cell count, differential, hemoglobin concentration, hematocrit, red blood cell count, and platelet count), blood biochemistry examination (aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, γ-glutamyltransferase, alkaline phosphatase, creatine phosphokinase, total bilirubin, direct bilirubin, total protein, albumin, blood urea, creatinine, uric acid, glucose, sodium, potassium, chloride, magnesium, calcium, and phosphorus, HbA1c (only at day 1)), and urinalysis (bilirubin, protein, glucose, ketone bodies, urobilinogen, occult blood, and sediment). Patients self-assessed their influenza symptoms and activities of daily living using an Influenza Symptom Severity scale (ISS) [0: none (normal), 1: mild (of little concern), 2:

moderate (very uncomfortable), 3: severe (intolerable)] for seven symptoms (cough, sore throat, headache, nasal stuffiness, feverishness or chills, muscle or joint pain, and fatigue) and a visual analogue scale (Influenza Impact Well-being Score [IIWS]) ranging from 0 (unable to perform usual activity at all) to 10 (able to perform all usual activity fully) (17). The questionnaire for this assessment was translated into three languages (Japanese, Korean, and Chinese). The ISS was assessed twice daily (morning and evening) from entry to day 8 and once daily (evening) from days 9 to 14. The IIWS was assessed once daily (evening) from entry to day 14. The results were recorded in a patient diary. Body temperature was measured four times daily (morning, noon, evening, and bedtime) from days 1 to 3 of treatment and twice daily (morning and evening) from days 4 to 14, and results were recorded in a patient diary.

A nasal swab from one naris and a single throat swab were collected at days 1 (baseline), 2 (optional), 3, and 8. All samples were taken from the same sites throughout the study. These samples were each transported in 3 mL viral transport medium to a central laboratory and divided for typing and gene sequencing using PCR (0.3 mL), virus titration (0.8 mL), and NA enzyme inhibitory assay (0.6 mL). Viral titers were calculated as $\log_{10}$ tissue culture infective dose $(TCID)_{50}$/mL of viral transport medium, according to the Spearman-Karber equation. Madin-Darby canine kidney (MDCK) cells were infected in triplicate with 0.1 mL of a 10-fold dilution series of samples (ranging from undiluted to $1:10^7$) in serum-free medium containing 3 μg/mL trypsin. Virus was adsorbed for 1 h, and cells were washed twice to remove unadsorbed virus and residual peramivir. MDCK cells were then incubated at 37°C in 5% CO_2 for 6 days. Following this incubation period, the appearance of cytopathic effect (CPE) on cell monolayers was scored using light microscopy, and the final titer was expressed as $TCID_{50}$/mL. When no CPE was observed using undiluted viral solution, this was defined as an undetectable level. We defined the undetectable level as $10^{0.5}$ $TCID_{50}$/mL. NA enzyme

inhibitory assays were performed on isolated virus using a standard fluorometric assay (18). The 50% inhibitory concentration (IC_{50}) was calculated by plotting the percent inhibition of NA activity versus the inhibitor concentration. A laboratory strain, A/PR/8/34 from the American Type Culture Collection, was also used as a standard strain in the NA inhibitory assay. The reliability of each assay was confirmed by the observation that the IC_{50} of peramivir trihydrate ranged from 0.2 to 2 nM for the standard strain. Results are reported as the mean ± standard deviation (SD) of three independent experiments. The sequences of the NA gene in A (H1N1) viruses isolated from patients on day 1 (baseline) were analyzed. cDNA was generated using viral RNA as a template and a PrimeScriptII 1st strand cDNA Synthesis kit, according to the manufacturer's instructions (TAKARA BIO INC.). The DNA fragment of a portion of the NA region was amplified from the cDNA with TaKaRa Ex Taq and PCR primers (forward: 5′-GAATTGGCTCCAAAGGAGATG-3′, reverse: 5′-GGGACGCGGGTTGTCACCGA-3′). The PCR products were purified, sequenced with a BigDye Terminator v3.1 Cycle Sequencing kit according to the manufacturer's instructions (Applied Biosystems), and analyzed on a DNA sequencer. Amino acid substitutions at positions 222, 234, 274, and 294 (N2 numbering) of the NA gene were investigated.

The plasma peramivir concentration was determined at the end of infusion of peramivir (day 1) and on day 3. When possible, the plasma concentration was also determined after the end of infusion (day 1) or on day 2. Blood samples were continuously collected from a subset of patients. The plasma concentration was measured as described (12). The lower limit for quantification of peramivir in plasma was 1.00 ng/mL.

Study outcomes

The primary efficacy endpoint was the time to alleviation of influenza symptoms. Alleviation of influenza symptoms was defined as the first time point when all seven

influenza symptoms (cough, sore throat, headache, nasal stuffiness, feverishness or chills, muscle or joint pain, and fatigue) were rated as “0: none” or “1: mild” for at least 21.5 h. In addition, the following secondary endpoints were assessed: (A) change from baseline in the composite symptom score, (B) proportion of patients whose body temperature returned to normal (<37.0°C), (C) time to resumption of usual activities (resumption of usual activities was defined as the first time point when IIWS was rated as “10: able to perform all usual activity fully”), (D) incidence of influenza-related complications (sinusitis, otitis media, bronchitis, and pneumonia), and (E) time-weighted change from baseline in virus titer.

The safety was evaluated by assessing the incidence of adverse events and adverse drug reactions. The severity was graded according to the DAIDS AE grading table (Division of AIDS table for grading the severity of adult and pediatric adverse events), and grades 1, 2, and 3 or higher corresponded to “mild,” “moderate,” and “severe,” respectively.

Statistical analysis

The primary efficacy analysis population was the intent-to-treat infected (ITTI) population and included all patients who had positive results with the RAT and received the study drug. Given the nature of the non-inferiority study, patients who were not treated as assigned were included in the analyses according to the actual treatment received, allowing a more conservative interpretation of results from the non-inferiority test.

The duration of influenza, the primary endpoint, was analyzed using a Cox proportional hazards model with the following covariates: the composite symptom score at baseline, current smoking behavior (yes/no), country (Japan/Korea/Taiwan), influenza virus type identified by RAT (type A/B/A and B), sex (male/female), presence of coexisting disease at baseline that was considered by the physician to be medically important and/or affecting evaluation (yes/no), and presence of any drugs received from the onset of influenza to

randomization (yes/no). The factors of sex, coexisting disease at baseline, and drugs received before randomization were added as covariates prior to unblinding because the blind review revealed that these factors may have affected the duration of influenza. The other covariates were used as minimization factors to ensure balance in randomization. Patients without alleviation of influenza symptoms were censored. The 97.5% confidence interval (CI) for the hazard ratio of the 300- and 600-mg groups compared to the oseltamivir group was calculated. Non-inferiority to the oseltamivir group was indicated if the upper confidence limit was less than 1.170. All statistical tests were performed at a two-sided significance level of 0.05 using Bonferroni adjustment for multiplicity. In addition, for each group, a Kaplan-Meier curve was plotted for the duration of influenza to calculate the median and 95% CI. In this study, the non-inferiority test was designed to show that peramivir was not inferior to oseltamivir by more than half of the difference between oseltamivir and placebo in terms of the log hazard ratio, and the corresponding non-inferiority margin was selected. The hazard ratio of oseltamivir versus placebo was estimated to be 0.73 based on the results of three previously reported studies (9, 16, 22). Accordingly, the non-inferiority margin was calculated to be −0.157 (= 0.5 × log{0.73}) in terms of the log hazard ratio and 0.170 (= (exp{−0.157} − 0.73)/0.73) in terms of the hazard ratio of peramivir versus oseltamivir.

Regarding the secondary endpoints, body temperature was summarized by calculating the summary statistics at each time point for each group and comparing between the groups at each time point with the van Elteren test, which was stratified by randomization factors. For time to resumption of activities, a Kaplan-Meier curve was plotted for each group to calculate the median and its 95% CI, and an analysis was performed using a Cox proportional hazards model with the randomization factors as covariates to estimate the difference between the groups. All statistical tests of these secondary endpoints were performed at a two-sided significance level of 0.05.

The time-weighted change in virus titer was compared between the groups with the van Elteren test, which was stratified by randomization factors. Patients with a positive virus titer at screening were included in the analysis. The virus titer was summarized by calculating the summary statistics at each time point for each group and comparing between the groups with the van Elteren test, which was stratified by randomization factors.

The target sample size of 1,050 patients (350 patients per group × 3 groups) was calculated to provide a power of 0.80 to detect a difference with a two-sided significance level of 0.025 in the non-inferiority test with a non-inferiority margin of 0.170 (peramivir versus oseltamivir). This calculation was based on the assumptions that the hazard ratios of peramivir and oseltamivir versus placebo were 0.67 and 0.73, respectively, and the duration of influenza was 73 h.

For the safety evaluation, reported adverse events and adverse drug reactions were summarized according to the MedDRA preferred terms (Ver. 11.1) to calculate the number of occurrences, number of affected patients, incidence, and 95% CI for each treatment group. The Clopper-Pearson method was used to calculate the CI of the percentage. In addition, the incidence was compared between groups using the Fisher's exact test.

All statistical analyses were performed using SAS Version 9.1 for Windows. Statistics were reported to one decimal place beyond the number of decimal places present in the original endpoint.

RESULTS

Study population

A total of 1,099 patients were randomly allocated to treatments (peramivir 300 mg, *n* = 366; peramivir 600 mg, *n* = 368; oseltamivir, *n* = 365; Figure 1). All patients were confirmed to be RAT positive prior to entry. Six patients who dropped out before treatment and two patients with no post-treatment efficacy data were excluded from all analyses. One patient who was randomized to the peramivir 600-mg group mistakenly received 300 mg peramivir and was thus included in the peramivir 300-mg group. Therefore, 1,091 patients (peramivir 300 mg, *n* = 364; peramivir 600 mg, *n* = 362; oseltamivir, *n* = 365) were included in the ITTI population, the primary efficacy analysis population. The three treatment groups did not differ significantly with respect to any baseline characteristics (Table 1).

A/H1N1 virus was isolated at baseline from 598 patients, and the base sequence of the NA gene was identified in 428 patients. The H274Y mutation (tyrosine instead of histidine at position 274 of the NA gene) was identified in 427 of 428 virus samples, and both R222Q and V234Y were identified in all samples. As shown in Table 2, the median IC_{50} for the A/H1N1 subtype at baseline was 100 nM (the upper limit of the assay) for oseltamivir and 21.59 nM for peramivir. However, the IC_{50} for A/H1N1 without H274Y at baseline was 0.661 nM for oseltamivir and 0.414 nM for peramivir.

Efficacy based on clinical symptoms

The median times to alleviation of symptoms were 78.0 (95% CI: 68.4, 88.6), 81.0 (72.7, 91.5), and 81.8 (73.2, 91.1) h in the 300-mg group, the 600-mg group, and the oseltamivir group, respectively (Table 3). The hazard ratios of the 300-mg and 600-mg groups compared to the oseltamivir group were 0.946 (97.5% CI: 0.793, 1.129) and 0.970 (0.814, 1.157),

respectively. The upper limits of both 97.5% CIs for the hazard ratios were less than the pre-specified non-inferiority margin. Both peramivir groups demonstrated non-inferiority to oseltamivir. The effect was consistent in subgroup analysis according to the influenza virus subtype (A/H1N1, A/H3N2, and B).

The proportion of patients whose body temperature returned to normal 24 h after treatment was significantly higher in the 300-mg and 600-mg groups (59.3% [213/359 patients] and 57.9% [209/361], respectively) than in the oseltamivir group (49.7% [181/364]; two-sided *p*-values = 0.0272 and 0.0326, respectively) (Figure 2).

The median times to resumption of usual activity were 155.7, 195.5, and 171.3 h in the 300-mg, 600-mg, and oseltamivir groups, respectively. Analysis using a Cox proportional hazards model found no significant difference between either peramivir group and the oseltamivir group.

Analysis of the incidence of physician-diagnosed influenza-related complications using Fisher's exact test found no significant difference between either peramivir group and the oseltamivir group [sinusitis: 1 case (0.3%) in the 300-mg group, 1 case (0.3%) in the 600-mg group, and 4 cases (1.1%) in the oseltamivir group; otitis media: 1 case (0.3%) in the 600-mg group; bronchitis: 6 cases (1.6%) in the 300-mg group, 6 cases (1.7%) in the 600-mg group, and 6 cases (1.6%) in the oseltamivir group; pneumonia: 3 cases (0.8%) in the 300-mg group, 1 case (0.3%) in the 600-mg group, and 2 cases (0.5%) in the oseltamivir group].

Virological efficacy

The mean virus titer ($\log_{10}$ $TCID_{50}$/mL) over time and the time-weighted change from baseline are shown in Figure 3 and Table 4, respectively. The time-weighted change from baseline in both peramivir groups was similar and numerically greater than that in the oseltamivir group. In the A/H3N2-infected subpopulation, the time-weighted change from

baseline in the 300-mg group was greater than that in the oseltamivir group (day 2, p = 0.0386; day 3, p = 0.0218). The proportions of virus-positive patients on days 2, 3, and 8 were 74.6% (150/201), 47.9% (162/338), and 1.2% (4/323), respectively, in the 300-mg group, 68.8% (132/192), 45.3% (158/349), and 1.5% (5/338), respectively, in the 600-mg group, and 82.1% (160/195), 49.9% (171/343), and 0.9% (3/331), respectively, in the oseltamivir group, decreasing over time in all groups. The proportion of virus-positive patients was lower in each of the peramivir groups, especially on day 2 in the 600-mg group. The proportion of virus-positive peramivir-treated patients was significantly lower than that of the oseltamivir group (p = 0.0038, compared with the Mantel-Haenszel test).

Safety

The incidence of adverse drug reactions (14.0%, 18.1%, and 20.0% in the 300-mg, 600-mg, and oseltamivir groups, respectively; Table 5) was significantly lower in the 300-mg group and non-significantly lower in the 600-mg group than in the oseltamivir group. Because peramivir required intravenous access, we were concerned that intravenous administration would result in injection-site adverse effects. As a result of the study, there were two cases of injection site anesthesia in the 300-mg group and one case of injection site irritation in the oseltamivir group. None of these adverse events were considered to be due to test drugs (instead, they were due to blood sampling after day 2 or concomitant drug administered intramuscularly).

Serious adverse events occurred in four patients (myalgia, bronchitis, influenza with acute exacerbation, and pneumonia) receiving 300 mg peramivir and two patients (pneumonia and vomiting) receiving oseltamivir. Only vomiting in the oseltamivir group was considered to be an adverse drug reaction.

Most adverse events were mild or moderate in severity. The incidences of severe adverse

events were 5.2%, 8.2%, and 6.6% in the 300-mg, 600-mg, and oseltamivir groups, respectively. There was no difference in the incidence of severe adverse events or adverse drug reactions among the treatment groups. The most common severe adverse events were prolonged QT and decreased neutrophil count. Prolonged QT was reported by five, eight, and 10 patients in the 300-mg, 600-mg, and oseltamivir groups, respectively. Because a separate thorough QT/QTc study showed that peramivir had no effect on the QT interval (unpublished data), the prolonged QT interval in our current study may have been due to variation in the QT interval during the course of infection. A decrease in neutrophil count was observed in four, nine, and nine patients in the 300-mg, 600-mg, and oseltamivir groups, respectively. In many of these patients, the lowest neutrophil count was observed on day 3. The grade and duration of the decreased neutrophil count observed in the previous Phase II study (12) and the current Phase III study are summarized in Table 6. The incidence of decreased neutrophil count in the peramivir groups was similar to that in the oseltamivir group. A decrease in neutrophil count also occurred in patients receiving a placebo in the Phase II study. The number of days that elapsed until recovery to Grade 1 indicated that recovery tended to be at least as rapid in the peramivir group as in the oseltamivir group.

Pharmacokinetics

The median duration of infusion was 0.47 h (range, 0.25–1.18 h). The median plasma peramivir concentrations at the end of infusion were 21,800 ng/mL (range, 4,010–43,500 ng/mL; $n = 328$) in the 300-mg group and 43,100 ng/mL (range, 18.6–94,900 ng/mL; $n = 317$) in the 600-mg group. The median plasma concentrations from 18 to 24 h after the end of infusion in the two groups were 17.4 (range, 3.31–315 ng/mL; $n = 153$) and 33.0 (range, 11.8–483 ng/mL; $n = 136$) ng/mL, respectively. The median plasma concentrations from 36 to 48 h after the end of infusion were 5.34 (range, 1.71–83.3 ng/mL, $n = 302$) and 10.6 (range,

3.59–51.1 ng/mL; *n* = 291) ng/mL, respectively. The time-plasma concentration plot is shown in Figure 4.

DISCUSSION

In this study, we compared the efficacy and safety of peramivir with those of oseltamivir (the most widely used anti-influenza drug) in patients with seasonal influenza virus infection. For the primary endpoint, the time to alleviation of symptoms and the non-inferiority of 300 mg and 600 mg peramivir to oseltamivir were demonstrated. In addition, peramivir significantly decreased the number of patients with fever, a secondary endpoint, on the day following administration as compared to oseltamivir, indicating a rapid peramivir effect. In terms of safety, the incidence of adverse drug reactions was lower in the peramivir groups than in the oseltamivir group and was significantly lower in the 300-mg group.

During the 2008–2009 influenza season, when this study was conducted, influenza A/H1N1 viruses (Russian strain) with the H274Y mutation were detected worldwide (5, 6, 15). In our current study as well, the H274Y mutant was isolated from nearly 100% of patients infected with the H1N1 virus. Given the possibility that oseltamivir may have been ineffective in the study population (approximately 50% of patients were infected with the less sensitive H274Y mutant of A/H1N1), the lack of a placebo group may have undermined the significance of the study. Kawai et al. found that the clinical efficacy of oseltamivir against the H1N1 virus with H274Y was reduced, especially among children (10, 11). In clinical trials with laninamivir (CS-8958) in the same season, this drug provided a shorter duration of influenza than that of oseltamivir in the H1N1 subpopulation in a pediatric trial, but not in an adult trial (20, 23). These results suggest that a reduction in the efficacy of oseltamivir against oseltamivir-resistant virus infection was not obvious in adult patients. In addition, the median duration of influenza in the oseltamivir group (81.8 h) in our current study was comparable to that in past clinical studies conducted in seasons when oseltamivir-sensitive strains were predominant (70.0–87.4 h for oseltamivir vs. 93.3–116.5 h for the placebo), suggesting that

the duration of influenza in our current study was within the range of year-to-year variation (9, 16, 22). Therefore, the clinical efficacy of oseltamivir was considered to have been maintained in this study involving adults, and the sensitivity of the study was thus ensured.

This study did not have a placebo group, and we could not confirm the clinical and virological efficacy of peramivir against the resistant A/H1N1 influenza virus that was wide-spread in the 2008-2009 season. All A/H1N1 viruses isolated in this study had both R222Q and V234Y mutations, which permit the evolution of H274Y-resistant virus by sustaining surface NA expression and the pathogenicity of the H274Y mutant virus was similar to that of oseltamivir-sensitive A/H1N1 virus (3, 5). The pandemic 2009 A/H1N1 (pH1N1) viruses that prevailed after the period of this present study remain mostly oseltamivir sensitive and introducing H274Y into the pH1N1 virus causes a large drop in total surface NA expression activity. Pizzorno et al. reported that the I222V mutation in the pH1N1 virus increases concern about the potential emergence and sustained communal transmission of resistant H274Y virus, and they argued the importance of continuous monitoring of antiviral resistance in clinical samples as well as the need to develop new drugs (19). The H274Y mutant A/H1N1 virus reportedly exhibits cross resistant to peramivir. The median IC_{50} values (min–max, limit of quantification: 100 nM) for the A/H1N1 virus in the present study were 21.59 (0.41–100.00) nM for peramivir and 100.00 (0.66–100.00) nM for oseltamivir, showing that the sensitivity to peramivir was less affected compared to oseltamivir. Because the plasma peramivir concentration immediately after the end of infusion of 300 or 600 mg was more than 20,000 ng/mL (ca. 60,000 nM) and much higher than the IC_{50} value, peramivir is expected to be effective even in patients infected with the resistant A/H1N1 virus (6, 13). Further study will be needed to clarify whether peramivir provides a clinical benefit to patients with the resistant virus that harbors the H274Y mutation.

In subgroups of patients infected with other subtypes (A/H3N2 and B), peramivir was as

effective as oseltamivir. The time to alleviation of symptoms was similar in both peramivir groups and the oseltamivir group for the subgroup of patients infected with influenza A/H3N2 virus and was significantly shorter (the upper limit of the 97.5% CI for the hazard ratio was lower than 1, Table 3) in the 300-mg group and non-significantly shorter in the 600-mg group than in the oseltamivir group for the subgroup of patients infected with influenza B virus. Thus, the consistent efficacy of a single intravenous dose of peramivir may provide reliable outcomes in the practical treatment of influenza.

Our results show that peramivir is generally safe and is expected to be consistently effective after single intravenous administration at a dose of 300 or 600 mg, regardless of the viral subtype, including A/H1N1, A/H3N2, and B influenza viruses. In addition, treatment with peramivir can be completed with a single intravenous dose, thus ensuring good compliance. These results highlight the usefulness of single-dose intravenous peramivir as an effective therapy for patients with seasonal influenza virus infection.

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TABLE 1. Baseline characteristics (intended-to-treat infected population)

	Peramivir 300 mg *n* = 364	Peramivir 600 mg *n* = 362	Oseltamivir *n* = 365
Region/country[a]-no. (%)			
Japan	247 (67.9)	249 (68.8)	246 (67.4)
Taiwan	81 (22.3)	79 (21.8)	84 (23.0)
Korea	36 (9.9)	34 (9.4)	35 (9.6)
Male sex-no. (%)	180 (49.5)	198 (54.7)	184 (50.4)
Age			
Mean (years) ± SD	34.9±11.7	35.9±12.0	34.6±11.7
Range (years)	20-78	20-78	20-80
Weight			
Mean (kg) ± SD	61.50±13.04	62.69±13.05	61.59±13.09
Range (kg)	39.5-120.0	33.4-104.7	40.0-140.1
Smoking[a]-no. (%)	113 (31.0)	111 (30.7)	112 (30.7)
Coexisting disease at baseline-no. (%)	127 (34.9)	146 (40.3)	132 (36.2)
Received drugs from onset of influenza to randomization-no. (%)	206 (56.6)	212 (58.6)	211 (57.8)
Influenza vaccination-no. (%)	64 (17.6)	56 (15.5)	63 (17.3)
Duration of influenza, no. (%)			
0–12 h	33 (9.1)	24 (6.6)	30 (8.2)
>12–24 h	129 (35.4)	117 (32.3)	131 (35.9)
>24–36 h	94 (25.8)	114 (31.5)	107 (29.3)
>36–48 h	108 (29.7)	106 (29.3)	95 (26.0)
>48 h	0 (0.0)	1 (0.3)	2 (0.5)
Composite symptom score[a], Mean ± SD	12.5±3.4	12.5±3.3	12.5±3.2
Body temp., Mean °C ± SD	38.53±0.49	38.48±0.49	38.56±0.52

Result of rapid antigen test[a], no. (%)			
A	335 (92.0)	333 (92.0)	338 (92.6)
B	27 (7.4)	29 (8.0)	25 (6.8)
A and B	2 (0.5)	0 (0.0)	2 (0.5)
Influenza virus subtype, no. (%)			
A/H1	197 (54.1)	200 (55.2)	201 (55.1)
A/H1, H3	0 (0.0)	0 (0.0)	1 (0.3)
A/H3	112 (30.8)	108 (29.8)	108 (29.6)
A/-	21 (5.8)	15 (4.1)	17 (4.7)
B	21 (5.8)	26 (7.2)	23 (6.3)
Unknown	13 (3.6)	13 (3.6)	15 (4.1)

[a]Randomization ensured balance for these factors.

TABLE 2. IC_{50} (nM) in NA inhibition assays of peramivir at baseline (intended-to-treat infected population)

Influenza virus subtype	Value for group			
	n	Peramivir	Oseltamivir	Zanamivir
A/H1	593			
Mean ± SD		22.25 ± 4.37	87.70 ± 16.38	1.35 ± 0.18
Median (Min-Max)		21.59 (0.41-100.00)	100.00 (0.66-100.00)	1.34 (0.97-3.41)
A/H3	323			
Mean ± SD		0.83 ± 0.17	0.63 ± 0.17	1.97 ± 0.37
Median (Min-Max)		0.82 (0.45-2.13)	0.62 (0.27-1.84)	1.91 (1.46-5.93)
B	70			
Mean ± SD		3.51 ± 0.39	16.53 ± 2.30	9.74 ± 1.10
Median (Min-Max)		3.58 (2.18-4.33)	16.77 (8.77-22.33)	9.79 (5.92-12.17)

Upper limit of the IC_{50} value was 100.0 nM.

The reliability of each assay was confirmed by the observation that the IC_{50} of peramivir trihydrate ranged from 0.2 to 2 nM for the standard strain (A/PR/8/34).

TABLE 3. Time to alleviation of symptoms (intended-to-treat infected population)

Population	Parameter	Value		
		Peramivir		Oseltamivir
		300 mg	600 mg	
Overall	*n*	364	362	365
	Median (h)	78.0	81.0	81.8
	95% CI	68.4, 88.6	72.7, 91.5	73.2, 91.1
	Hazard ratio[a]	0.946	0.970	---
	97.5% CI	0.793, 1.129[b]	0.814, 1.157[b]	---
A/H1	*n*	197	200	201
	Median (h)	80.2	83.6	88.8
	95% CI	69.3, 90.6	72.7, 101.9	73.1, 102.2
	Hazard ratio[a]	0.854	0.927	---
	97.5% CI	0.672, 1.085	0.730, 1.176	---
A/H3	*n*	112	108	108
	Median (h)	69.9	70.6	75.1
	95% CI	54.4, 97.1	47.7, 91.9	63.4, 92.6
	Hazard ratio[a]	1.039	0.958	---
	97.5% CI	0.745, 1.448	0.687, 1.335	---
B	*n*	21	26	23
	Median (h)	55.3	92.8	92.7
	95% CI	43.9, 86.4	57.4, 116.1	70.2, 138.5
	Hazard ratio[a]	0.445	0.706	---
	97.5% CI	0.202, 0.982	0.341, 1.460	---

[a]Hazard ratios compared to the oseltamivir group were estimated using Cox proportional hazards models, which were adjusted for current smoking behavior, composite symptom score at baseline, country/region, influenza virus type, sex, complications, and previous therapy.

[b]Both peramivir groups were non-inferior to the oseltamivir group with a non-inferiority margin of 0.170.

TABLE 4. Time-weighted change from baseline in virus titer ($TCID_{50}$/mL) (intended-to-treat infected population)

Population	Parameter	Value		
		Peramivir		Oseltamivir
		300 mg	600 mg	
Overall				
From day 1 to day 2	*n*	201	192	195
	Mean ± SD	−1.10 ± 0.90	−1.08 ± 0.82	−1.04 ± 0.84
	P value[a]	0.4278	0.2252	---
From day 1 to day 3	*N*	338	349	343
	Mean ± SD	−1.71 ± 1.21	−1.71 ± 1.10	−1.63 ± 1.11
	P value[a]	0.1337	0.1778	---
From day 1 to day 8	*N*	323	338	331
	Mean ± SD	−2.97 ± 1.53	−2.91 ± 1.44	−2.82 ± 1.49
	P value[a]	0.0674	0.2066	---
A/H1				
From day 1 to day 2	*N*	115	117	111
	Mean ± SD	−1.18 ± 0.95	−1.15 ± 0.90	−1.06 ± 0.97
	P value[a]	0.6244	0.4678	---
From day1 to day 3	*N*	190	198	195
	Mean ± SD	−1.79 ± 1.26	−1.81 ± 1.19	−1.71 ± 1.22
	P value[a]	0.5092	0.5204	---
From day 1 to day 8	*N*	182	191	187
	Mean ± SD	−3.20 ± 1.55	−3.17 ± 1.42	−3.04 ± 1.57
	P value[*]	0.1735	0.4007	---
A/H3				
From day 1 to day 2	*N*	58	54	60
	Mean ± SD	−1.23 ± 0.68	−1.12 ± 0.52	−1.01 ± 0.60
	P value[a]	0.0386	0.3129	---

From day 1 to day 3	*N*	106	105	107
	Mean ± SD	−1.87 ± 0.91	−1.68 ± 0.71	−1.58 ± 0.76
	P value[a]	0.0218	0.2434	---
From day 1 to day 8	*N*	102	103	103
	Mean ± SD	−2.86 ± 1.28	−2.57 ± 1.05	−2.48 ± 1.01
	P value[a]	0.0644	0.5459	---
B				
From day 1 to day 2	*N*	13	10	16
	Mean ± SD	−0.82 ± 1.02	−1.13 ± 0.93	−1.21 ± 0.73
	P value[a]	0.1612	0.8342	---
From day 1 to day 3	*N*	21	25	23
	Mean ± SD	−1.68 ± 1.26	−2.08 ± 1.13	−2.08 ± 1.00
	P value[a]	0.1891	0.6794	---
From day 1 to day 8	*N*	20	24	23
	Mean ± SD	−3.46 ± 1.31	−3.92 ± 1.61	−3.99 ± 1.24
	P value[*]	0.1128	0.8778	---

[a]P values were determined with the van Elteren test, which was stratified by current smoking behavior, composite symptom score at baseline, and country/region.

TABLE 5. Summary of adverse events (AEs) and adverse drug reactions (ADRs) (safety population)

Parameter	Values		
	Peramivir		Oseltamivir (*n* = 365)
	300 mg (*n* = 364)	600 mg (*n* = 364)	
Number of AEs	272	288	297
Number of patients with ≥1 AE (%)	170 (46.7)	174 (47.8)	178 (48.8)
95% CI (%)	41.5, 52.0	42.6, 53.1	43.5, 54.0
P value[a]	0.6040	0.8242	-
Number of mild AEs	90	90	95
Number of patients with ≥1 mild AE (%)	69 (19.0)	66 (18.1)	74 (20.3)
Number of moderateAEs	161	166	177
Number of patients with ≥1 moderate AE (%)	119 (32.7)	116 (31.9)	121 (33.2)
Number of severe AEs	21	32	25
Number of patients with ≥1 severe AE (%)	19 (5.2)	30 (8.2)	24 (6.6)
Number of ADRs	80	99	104
Number of patients with ≥1 ADR (%)	51 (14.0)	66 (18.1)	73 (20.0)
95% CI (%)	10.6, 18.0	14.3, 22.5	16.0, 24.5
P value[a]	0.0382	0.5718	-
Number of mild ADRs	40	42	48
Number of patients with ≥1 mild ADRs (%)	29 (8.0)	32 (8.8)	40 (11.0)
Number of moderate ADRs	37	47	47
Number of patients with ≥1 moderate ADRs (%)	29 (8.0)	34 (9.3)	37 (10.1)
Number of severe ADRs	3	10	9

Number of patients with ≥1 severe ADRs (%)	3 (0.8)	10 (2.7)	9 (2.5)
AEs (>3% in either group)			
Neutrophil count decreased	39 (10.7)	38 (10.4)	34 (9.3)
Diarrhoea	24 (6.6)	30 (8.2)	27 (7.4)
Protein urine present	17 (4.7)	16 (4.4)	22 (6.0)
Blood glucose increased	11 (3.0)	14 (3.8)	12 (3.3)
WBC urine positive	14 (3.8)	8 (2.2)	16 (4.4)
Nausea	8 (2.2)	8 (2.2)	20 (5.5)
Vomiting	2 (0.5)	6 (1.6)	15 (4.1)
ADRs (>3% in either group)			
Diarrhoea	14 (3.8)	20 (5.5)	19 (5.2)
Neutrophil count decreased	9 (2.5)	14 (3.8)	13 (3.6)
Nausea	2 (0.5)	7 (1.9)	16 (4.4)

[a]P values were calculated by intergroup comparison between the peramivir and oseltamivir groups using Fisher's exact test.

TABLE 6A Summary of post-baseline minimum neutrophil count by grade (safety population)

	Phase II study[a]		Phase III study[b]	
	Peramivir *n* = 198	Placebo *n* = 100	Peramivir *n* = 723	Oseltamivir *n* = 363
Grade 0 (≥1300/μL)	151 (76.3%)	89 (89.0%)	557 (77.0%)	288 (79.3%)
Grade 1 (≥1000, <1300/μL)	29 (14.6%)	7 (7.0%)	93 (12.9%)	41 (11.3%)
Grade 2 (≥750, <1000/μL)	14 (7.1%)	3 (3.0%)	60 (8.3%)	25 (6.9%)
Grade 3 (≥500, <750/μL)	3 (1.5%)	1 (1.0%)	10 (1.4%)	7 (1.9%)
Grade 4 (<500/μL)	1 (0.5%)	0 (0.0%)	3 (0.4%)	2 (0.6%)

[a]"Phase II study" is a placebo-controlled randomized study (already published (12)).

[b]"Phase III study" is an oseltamivir-controlled randomized study (our present study).

TABLE 6B Summary of time (duration) to recovery from Grade 2 or more severe neutropenia to Grade 1 or less (safety population)

	Phase II study[a]		Phase III study[b]	
	Peramivir *n* = 198	Placebo *n* = 100	Peramivir *n* = 723	Oseltamivir *n* = 363
Number of affected patients	18	4	73	34
1 day	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
2-3 days	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
4-9 days	0 (0.0%)	0 (0.0%)	66 (90.4%)	25 (73.5%)
10-14 days	14 (77.8%)	2 (50.0%)	4 (5.5%)	6 (17.6%)
≥15 days	4 (22.2%)	2 (50.0%)	3 (4.1%)	1 (2.9%)
Not recovered	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (5.9%)[c]

Duration (day) = (date of recovery to Grade 1 or less) − (date of onset of Grade 2 or more severe) + 1

[a]"Phase II study" is a placebo-controlled randomized study (already published (12)).

[b]"Phase III study" is an oseltamivir-controlled randomized study (our present study).

[c]Not recovered because follow-up was discontinued.

Figure Legends

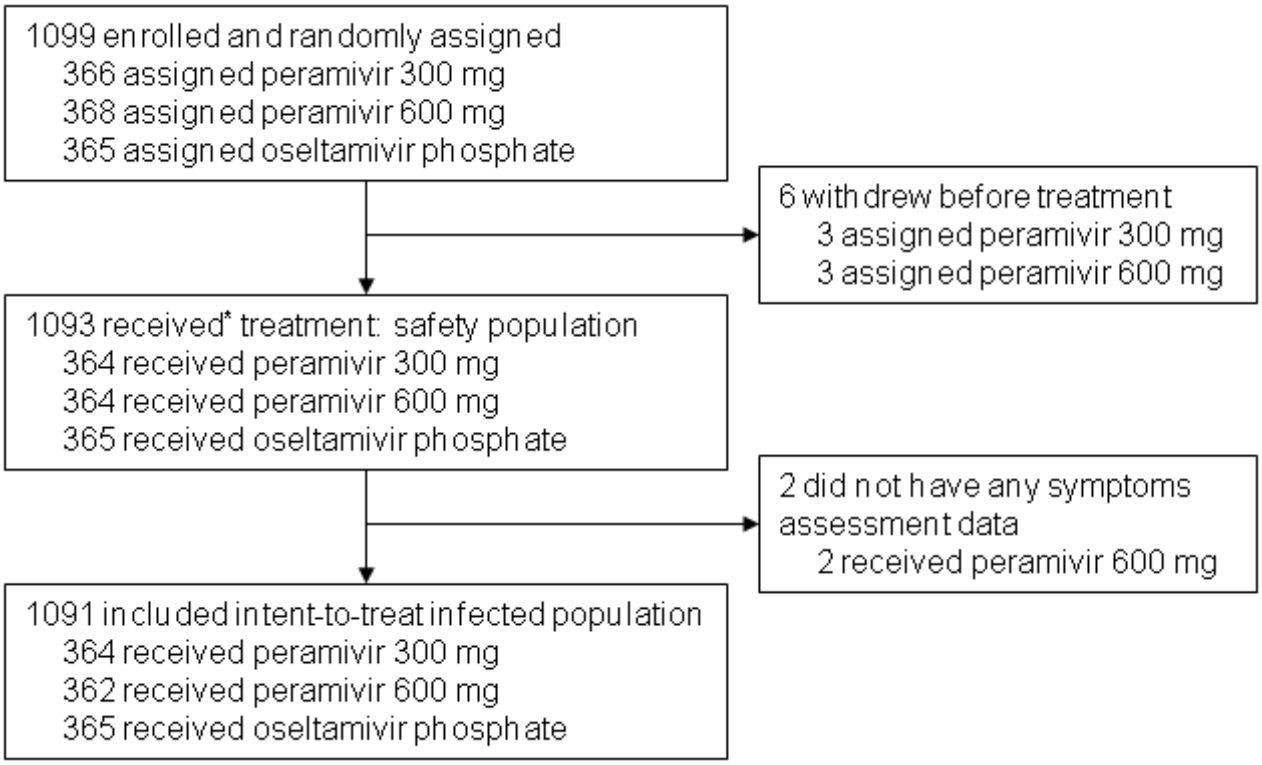


FIG. 1. Study profiles.

*, One patient assigned to the 600-mg group received 300 mg peramivir. This patient was included in the actually administered treatment.

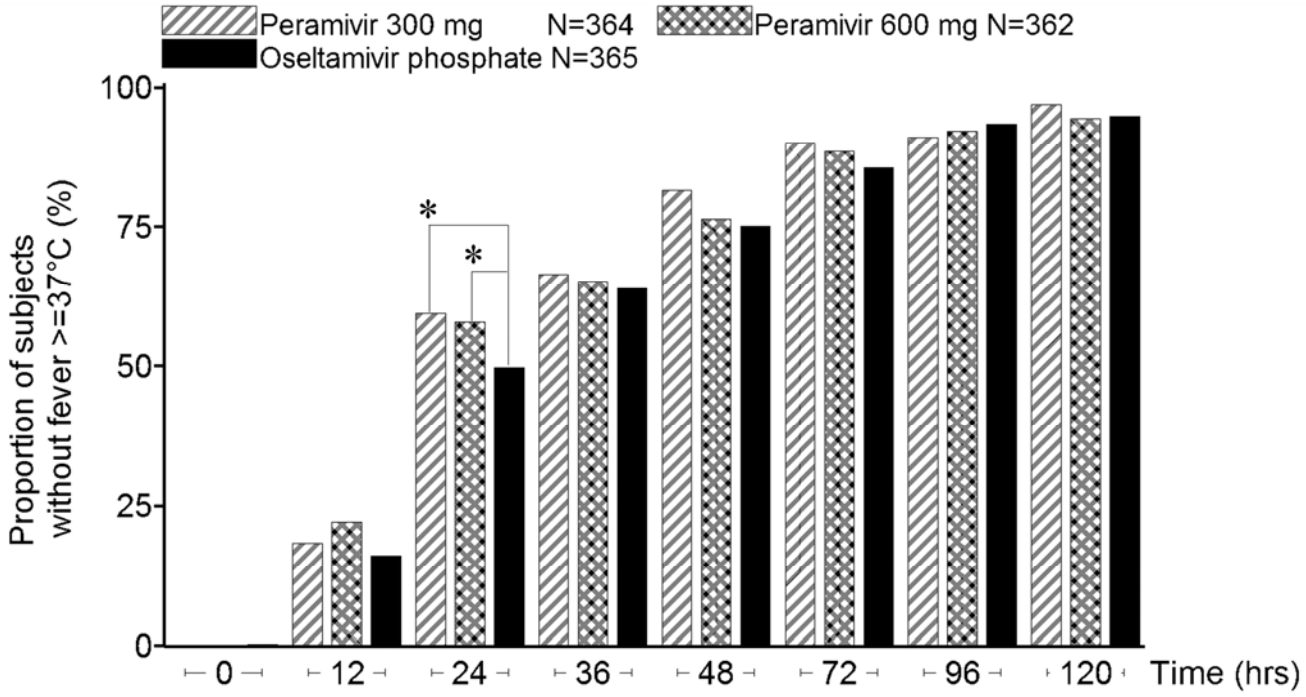


FIG. 2. Proportion of patients reporting normal temperature (intent-to-treat infected population).

*, $p < 0.05$ between peramivir and oseltamivir as determined by the Mantel-Haenszel test, which was stratified by current smoking behavior, composite symptom score at baseline, country/region, and influenza virus type

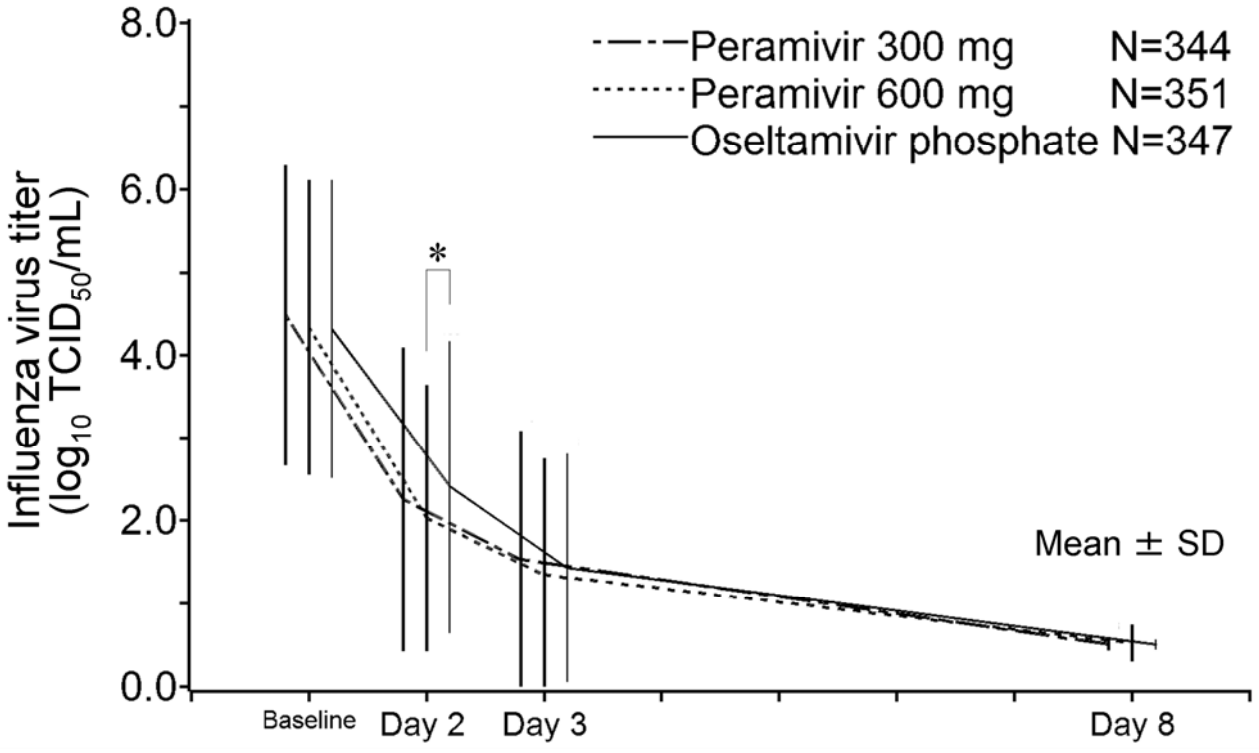


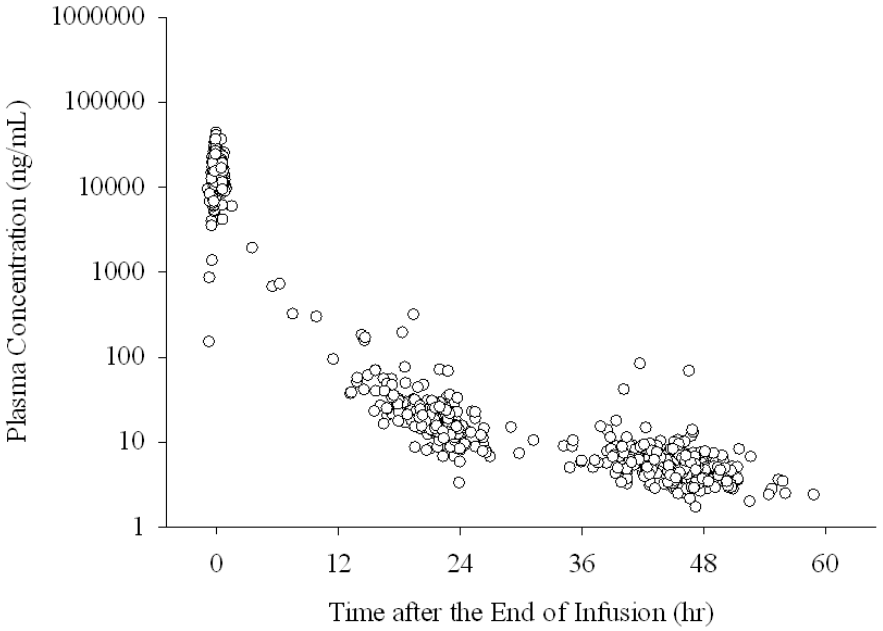
FIG. 3. Mean and SD of influenza virus titers (log_{10} $TCID_{50}$/mL) over time (intent-to-treat infected population).

The data analyzed were from the subset of patients who were positive for the influenza virus at baseline.

Virus titers below the lower limit of quantification were set to 0.5.

*, $p < 0.05$ between peramivir and oseltamivir as determined by the van Elteren test, which was stratified by current smoking behavior, composite symptom score at baseline, country/region, and influenza virus type.

A.



B.

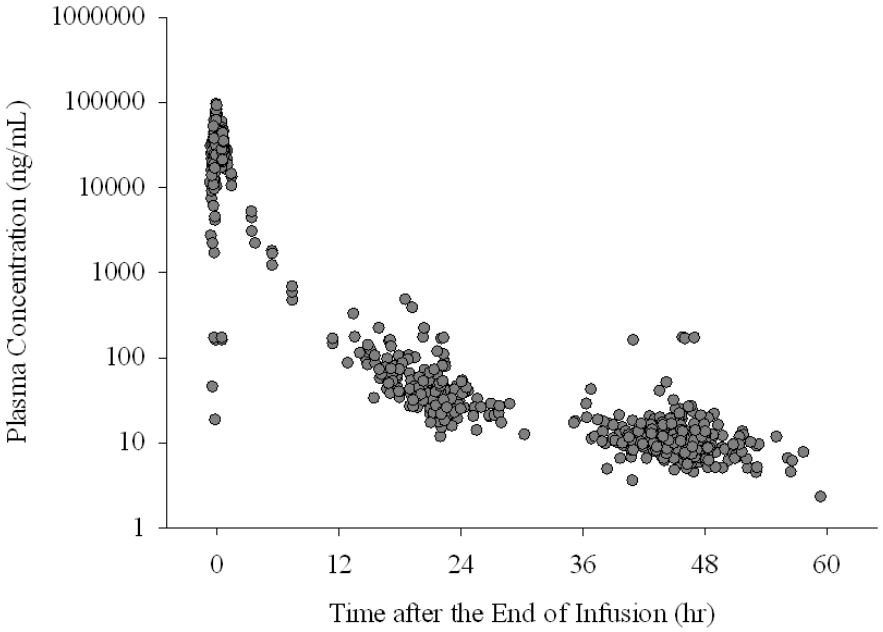


FIG 4. Plasma concentration of peramivir

A, 300 mg peramivir; B, 600 mg peramivir.

The conversion factor between the plasma concentration (ng/mL) and the IC_{50} (nM) was as follows: 1 ng/mL = 2.84 nM.