Zanamivir, at 600 mg Twice Daily, Inhibits Oseltamivir–Resistant 2009 pandemic H1N1 Influenza Virus in an in vitro Hollow Fiber Infection Model System

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Abstract

In 2009, a novel H1N1 influenza A virus emerged that spread worldwide initiating a pandemic. Various isolates obtained from disparate parts of the world were shown to be uniformly resistant to the adamantanes, but sensitive to neuraminidase inhibitors, oseltamivir and zanamivir. Over time, resistance to oseltamivir became more prevalent among pandemic H1N1 virus isolates while most of these H1N1 isolates remained susceptible to zanamivir. The government has proposed the use of intravenous (i.v.) zanamivir to treat serious influenza virus infections among hospitalized patients. To use zanamivir effectively in patients with severe influenza, it is necessary to know the optimal dose and schedule of administration of zanamivir that will inhibit the replication of oseltamivir-sensitive and resistant influenza viruses. Therefore, we performed studies using the in vitro hollow fiber infection model system to predict optimal dosing regimens for zanamivir against an oseltamivir-sensitive and an oseltamivir-resistant virus. Our results demonstrated that zanamivir, at a dose of 600 mg given Q12, inhibited the replication of oseltamivir-sensitive and oseltamivir-resistant influenza viruses throughout the course of the experiment. Thus, our findings suggest that intravenous zanamivir, at a dose of 600 mg Q12, could be used to treat hospitalized patients suffering from serious oseltamivir sensitive or resistant influenza virus infections.
Introduction

In April, 2009, the Center for Disease Control and Prevention (CDC) identified a novel swine origin influenza A (H1N1) virus that was obtained from two pediatric cases of influenza in California (10, 19, 27, 28, 40). Both of these isolates were strains of a reassorted influenza A virus composed of the PB2 and PA gene segments of North American lineage avian influenza virus, the PB1 gene segment of seasonal H3N2 human influenza virus, the HA, NP, and NS gene segments from classic North American swine influenza virus, and the NA and M gene segments from Eurasian swine influenza virus. Subsequently, this novel swine-origin influenza virus was also isolated from patients in Argentina, Canada, Chile, Mexico and New York City (1, 2, 9, 14, 17, 19, 22, 32, 34, 46, 55). The virus rapidly spread throughout the world suggesting that it is easily transmitted from person to person. In July 2009, the World Health Organization declared a pandemic due to infection with this novel H1N1 influenza virus (54). In this report, these pandemic H1N1 influenza viruses will be referred to as pH1N1 influenza viruses.

This new strain of pH1N1 influenza virus caused disease in all age groups with an unusual spike of disease in people 5 to 49 years of age. Previously healthy teenagers and young adults were particularly susceptible to infection that occasionally led to serious life-threatening disease (22, 33). This demographic is reminiscent of the 1918-1919 influenza pandemic where young, healthy adults were killed by the infection whereas the elderly were spared. It is estimated that more than 40 million people died throughout the world from the 1918-1919 influenza pandemic (30). These comparative demographics caused much concern among federal, state and local public health officials since they feared that a major influenza pandemic similar to the 1918-1919 pandemic was upon us. Fortunately, to date, the pandemic has caused much morbidity but it has not proven as deadly as the 1918-1919 pandemic with an overall
fatality rate of less than 1% of those infected with laboratory confirmed strains of the pH1N1 influenza virus (22, 53). The vast majority of those infected with pH1N1 influenza virus recovered with no specific treatment or, if treated within the first 48 hr of initial symptoms with oseltamivir or zanamivir, experienced a full recovery sooner than those patients without treatment. However, the situation was different for pregnant and postpartum women (35) and seriously ill patients in intensive care units, where many of these patients died from their disease (34, 53). After a year of worldwide circulation, the vast majority of pH1N1 viral strains remain genetically stable and susceptible to the neuraminidase inhibitors, oseltamivir and zanamivir. An effective vaccine is available for the prevention of pH1N1 influenza (8).

Drug susceptibility studies of clinical isolates of pH1N1 influenza viruses show that they are uniformly resistant to amantadine and rimantadine but most isolates are susceptible to oseltamivir and essentially all are susceptible to zanamivir (7, 8). Clinical isolates that are susceptible to oseltamivir are also susceptible to the experimental neuraminidase inhibitor, peramivir, which has been licensed for emergency use as an intravenous injection in cases of serious influenza (3). Although numerous examples of oseltamivir-resistant pH1N1 influenza viruses have been reported (12, 13, 16, 21, 38, 39, 42, 50), to date there have been few examples of zanamivir-resistant pH1N1 influenza virus or H3N2 seasonal influenza virus isolated from patients (38).

It is well established that zanamivir, given as an aerosol, is effective for the treatment of uncomplicated influenza (24). Additionally, intravenous zanamivir is well tolerated in people (6) and is effective in the clinic for patients infected with oseltamivir-resistant H3N2 influenza virus (15, 20, 23, 26). Little is known of the pharmacodynamics of zanamivir (6). With the increase in the number of reports of oseltamivir-resistant pH1N1 influenza virus clinical isolates and the
paucity, so far, of reports of zanamivir-resistant pH1N1 influenza virus clinical isolates,
zanamivir may be the drug of choice for intravenous therapy for hospitalized patients with
serious influenza, particularly those infected with oseltamivir-resistant influenza viruses.

To use zanamivir in the clinical setting, it is important to determine the optimal dose and
schedule of administration (pharmacodynamics) of zanamivir for oseltamivir-susceptible and
oseltamivir-resistant pH1N1 influenza viruses. To that end, we used our in vitro hollow fiber
infection model (HFIM) system to performed dose range and dose fractionation studies for
zanamivir on a wild type H1N1 influenza virus, A/Mexico/4108/2009, and on an oseltamivir-
resistant H1N1 influenza virus, A/Hong Kong/2369/2009 [H275Y]. Our results demonstrate that
zanamivir, given twice a day at the recommended exposure equivalent to a dose of 600 mg (1200
mg daily) suppresses A/Mexico and A/Hong Kong virus replication over a five day period in our
HFIM system. These results support the plan to use intravenous administration of zanamivir for
the treatment of serious influenza.
Materials and Methods

Cells and viruses. MDCK cells (ATCC #CCL-34) were obtained from the American Type Culture Collection (Manassas, VA) and propagated as described (5, 37). The 2009 pandemic influenza viruses A/New York/18/2009, A/Mexico/4108/2009, A/California/04/2009, and A/HongKong/2369/2009 [H275Y] were obtained from the Center for Disease Control and Prevention in Atlanta, GA. Virus stocks were prepared and stored at –80°C as described previously (5, 37).

Antiviral drugs. Zanamivir, peramivir, and oseltamivir were obtained in powdered form from GlaxoSmithKline (Durham, UK), Biocryst Pharmaceuticals (Birmingham, AL) and Roche Pharmaceuticals (Basel, Switzerland), respectively. All drug stocks were prepared in sterile double distilled water to yield a final concentration of 10 mg/ml of active compound. Drug solutions were then filter-sterilized through a 0.2 micron filter and dispensed in small volumes. Peramivir and zanamivir aliquots were frozen at -80°C and oseltamivir aliquots were stored at 4°C.

EC$_{50}$ determination. The antiviral activities of the neuraminidase inhibitors, oseltamivir, peramivir, and zanamivir, on pH1N1 influenza viral clinical isolates were determined using the commercially available NA-Star® Influenza Neuraminidase Inhibitor Resistance Detection Kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. The concentration of drug that was required to reduce the neuraminidase activity by 50% when compared to no-treatment controls was calculated as described below. The mean effective concentration 50 (EC$_{50}$) values and the standard deviation between experiments for each virus/drug combination are reported for three independent experiments.
Dose range and dose fractionation studies in the HFIM system. To determine the optimal dose and schedule of administration of zanamivir that inhibits the production of pHIN1 A/Mexico (wild type) and pH1N1 A/Hong Kong (oseltamivir-resistant) influenza viruses, we employed the HFIM system as previously described (5, 37). In brief, hollow fiber systems (FiberCell Systems, Inc, Fredrick, MD) containing $10^2$ virus-infected MDCK cells and $10^8$ uninfected MDCK cells in virus growth medium (VGM) [MEM, 0.2% final concentration of bovine serum albumin (BSA) (Sigma-Aldrich, Inc; St. Louis, MO), 2 µg/ml of L-1-(tosyl-amido-2-phenyl)ethyl chloromethyl ketone (TPCK)-treated trypsin (Sigma-Aldrich, Inc), 100 U/ml of penicillin, and 100µg/ml of streptomycin] were incubated at 36°C, 5% CO$_2$. The concentration-time profiles simulated for zanamivir in the hollow fiber cartridges were extrapolated from human serum pharmacokinetic data obtained from the Zanamivir Investigator’s Brochure provided by GlaxoSmithKline (56). In these studies, pharmacokinetic profiles for zanamivir at 1200 mg, 600 mg, and 300 mg administered once a day (Q24h) and 600 mg, 300 mg, and 150 mg given twice a day (Q12h) were simulated over a 5 day period. Zanamivir was removed from the system at a rate simulating a 2.5 hr half-life, the mean human half-life described in the Zanamivir Investigator’s Brochure for intravenous zanamivir (56). Each hollow fiber unit was sampled daily and the effect of the antiviral drugs on virus replication was determined by plaque assay of released virus.

Zanamivir concentrations in the HFIM system. To determine the actual concentration of zanamivir in the medium in each hollow fiber unit, a sample was removed daily from the central reservoir of each hollow-fiber unit. Media samples were diluted with HPLC water (0.050mL sample into 1.0mL water), and analyzed by high pressure liquid chromatography tandem mass spectrometry (LC/MS/MS) for zanamivir concentrations. The LC-MS-MS system was
comprised of a Shimadzu Prominence HPLC system and an Applied Biosystems/MDS Sciex API5000 LC-MS-MS system. Chromatographic separation was performed using a Thermo Scientific Hypersil Gold C-18 column 150 x 4.6 mm, 5 µm column and a mobile phase consisting of 90% 10 mM ammonium acetate and 10% acetonitrile at a flow rate of 0.75 mL/min. Zanamivir concentrations were obtained using LC/MS/MS monitoring the MS/MS transition m/z 333 → m/z 60. Analysis run time was 3.5 minutes. The assay was linear over a range of 0.05 – 20.0 µg/ml (r² > 0.997). The inter-day CVs for the quality control samples analyzed in replicates of three at three concentrations (0.100, 1.00, and 10.0 µg/mL) on each analysis day were 6.17% or less with accuracies (%REC) ranging between 103% to 106% .

**Statistical analysis.** An inhibitory sigmoid-$E_{\text{max}}$ (maximal effect) model of the form \( \text{effect} = \text{control effect} - \left[ E_{\text{max}} \times \text{exposure}^H / (\text{exposure}^H + EC_{50}^H) \right] \) was fit to the data. Control effect is the measured viral output in the absence of drug, $E_{\text{max}}$ is the greatest reduction in viral output produced by drug exposure, and $H$ is Hill's constant. The model was fit to the data by nonlinear regression analysis, performed within the ADAPT II package of programs (11).

The impact of both dose (blocking factor) and schedule (covariate) were examined by taking the trapezoidal rule Area Under the Curve (AUC) of viral burden from baseline to 120 hours and analyzing the impact with ANalysis Of VAriance (SYSTAT for Windows, v 11).
Results

EC\textsubscript{50} values of anti-influenza virus drugs for pH1N1 strains of influenza virus. The susceptibilities of four 2009 pH1N1 influenza virus strains to three different neuraminidase inhibitors (oseltamivir, peramivir, and zanamivir) were evaluated by measuring the inhibitory effect of each drug on the neuraminidase activity of each virus (Table 1). The wild type strains A/California, A/New York, and A/Mexico were highly sensitive to oseltamivir, peramivir, and zanamivir, as the EC\textsubscript{50} values reported for all three compounds were below 0.1 ng/ml. In contrast, the mutant A/Hong Kong [H275Y] strain had EC\textsubscript{50} values of 16.45 ng/ml for oseltamivir and 3.065 ng/ml for peramivir. These results indicate that A/Hong Kong [H275Y] is approximately 200-fold and 85-fold more resistant to oseltamivir and peramivir, respectively, when compared to wild type strains. The EC\textsubscript{50} value for zanamivir was 0.05 ng/ml for the mutant A/Hong Kong [H275Y] strain, a value very similar to those reported for the wild type strains (Table 1). Additionally, we performed viral burden drug susceptibility assays to test the effect of amantadine, an anti-influenza drug that blocks the M2 channel, on viral replication. All four pH1N1 influenza strains were resistant to amantadine (data not shown), which is consistent with previously published reports (7, 8). Overall, these results demonstrate that all pH1N1 influenza viruses tested here are resistant to amantadine but remain susceptible to neuraminidase inhibitors in our studies. The H275Y mutation confers resistance to both oseltamivir and peramivir, but does not alter susceptibility to zanamivir.

Growth of influenza viruses in the hollow fiber infection model system. The HFIM system is used to determine the pharmacodynamics of antiviral compounds for viruses grown in tissue culture cells (5, 37). We employed two of these four viruses, A/Mexico (wild type, oseltamivir susceptible) and A/Hong Kong [H275Y] (oseltamivir-resistant), in our HFIM system to elucidate...
the optimal dose and schedule of administration (pharmacodynamics) of zanamivir that will
inhibit virus replication. Based on our previous experience with H3N2 seasonal influenza virus
in the HFIM system (5), we have determined that hollow fiber cartridges containing $10^2$
influenza virus-infected MDCK cells and $10^8$ uninfected MDCK cells yield optimal replication
kinetics with peak viral titers occurring 48 hrs after inoculation into the HFIM system. To
ensure that these conditions were favorable for pH1N1 influenza virus strains, we performed a
virus growth curve study with the above mentioned strains in the HFIM system. Two hollow
fiber cartridges were inoculated with either $10^2$ A/Mexico-infected MDCK cells or $10^2$ A/Hong
Kong [H275Y]-infected MDCK cells each set of virus-infected cells was mixed with $10^8$
uninfected MDCK cells. Each unit was continuously infused with VGM for 5 days. At various
times post infection, the extracapillary space (ECS) of each hollow fiber unit was sampled and
the amount of infectious virus released into the medium was determined by plaque assay. The
data in Figure 1 show that in both cases, infectious virus was produced over the five day period
with a peak in virus production of around $10^7$ PFU/ml at 48 hr post infection followed by a slow
decline in the amount of infectious virus over the 120 hour time course of the experiment. This
decline in virus infectivity is most likely due to the lack of fresh target cells to keep the infection
going and the temperature sensitivity of virus infectivity (5, 43). The data clearly demonstrated
that the pH1N1 A/Mexico and pH1N1 A/Hong Kong [H275Y] strains of influenza virus can
replicate to high titers in MDCK cells in the HFIM system.

The effect of zanamivir on the replication of wild type pH1N1 A/Mexico influenza virus in
the HFIM system. We assessed the inhibitory effect of zanamivir on the replication of the wild
type strain of pH1N1 influenza virus, A/Mexico, in the HFIM system. In this study, we
inoculated two hollow fiber cartridges each with $10^2$ A/Mexico-infected MDCK cells and $10^8$
uninfected MDCK cells. One unit was continuously infused with VGM without zanamivir and served as a no-treatment control. The other unit received a 600 mg exposure of zanamivir on a twice daily (Q12h) schedule. This is the clinically recommended dosing regimen for intravenous administration of zanamivir. The human half-life of 2.5 hrs was simulated in these experiments. The measured zanamivir concentrations were within 10% of the target values, demonstrating that the desired concentration time profiles were achieved in this experiment (data not shown).

Zanamivir suppressed viral growth by nearly 100-fold at 24 hr when compared to the no-treatment control (Fig. 2). By 48 hr, viral burden peaked in both experimental arms, but the amount of virus was about 10-fold lower in the zanamivir treatment arm (control arm = 1.9 x 10^7 PFU/ml; zanamivir arm = 2.8 x 10^6 PFU/ml). Zanamivir continued to inhibit A/Mexico virus replication for the duration of the study. These results suggest that intravenous zanamivir is an effective treatment option against wild type pH1N1 influenza virus at an exposure of 600 mg Q12h.

Dose range and dose fractionation study of zanamivir for A/Hong Kong [H275Y] influenza virus in the hollow fiber system. Because intravenous zanamivir will likely be administered to patients with complicated oseltamivir-resistant influenza infections, we performed a more comprehensive examination of the antiviral activity of zanamivir against the oseltamivir-resistant pH1N1 A/Hong Kong [H275Y] influenza virus strain in the HFIM system. As stated above, the clinically recommended dosing regimen for intravenous zanamivir is 600 mg Q12h. Although this dose has been shown to be well tolerated in patients (6), we wanted to determine if once-a-day administration and/or lower doses of zanamivir could achieve similar levels of treatment success. Therefore, we performed a dose range and dose fractionation study with zanamivir against A/Hong Kong [H275Y] in the HFIM system. In these studies, average exposures for
zanamivir at 1200, 600, or 300 mg administered once a day (Q24h) or average exposures at 600, 300, or 150 mg administered twice a day (Q12h) were simulated in hollow fiber units containing MDCK cells infected with the A/Hong Kong [H275Y] strain of influenza virus. The drug concentrations in the HFIM system from the above simulated exposures were below the known cellular cytotoxicity levels for zanamivir in MDCK cells (48). All doses were followed by a no-drug wash out to simulate the human half life of 2.5 hr. In the absence of drug, the A/Hong Kong [H275Y] virus grew well with a peak in virus replication at 48 hr followed by a gradual decline over time (Fig. 3). The simulated exposures of 1200, 600 and 300 mg given once a day (Q24h) equally suppressed viral replication at 24 hr post infection, as viral titers were approximately 100-fold lower compared to the control (Fig. 3A). By 48 hrs, viral load increased in the treatment arms but still remained 4-fold lower than the control. At the later time points, all doses yielded similar results and suppressed viral growth relative to the control by a factor of 10. The 600, 300, and 150 mg Q12h simulated zanamivir regimens suppressed viral replication better than the equivalent exposures given Q24h (Fig. 3B). These Q12h dosage regimens resulted in a sustained dose response throughout the course of the infection. The 600 mg Q12h dose yielded maximal suppression of virus replication at all time points tested, suppressing viral replication by 1000-fold at 24 hrs, 100-fold at 48 hrs, and 10-fold at the remaining time points. These results demonstrate that zanamivir can inhibit the replication of the oseltamivir-resistant pH1N1 A/Hong Kong [H275Y] in MDCK cells. Although 150 mg Q12h and 300 mg Q12h regimens inhibited viral burden throughout the course of infection, the 600 mg Q12h exposure clearly provided better suppression. Additionally, the Q12h regimens inhibit viral replication to a greater extent when compared to the Q24h regimens, suggesting that the
pharmacodynamically-linked index for zanamivir for the A/Hong Kong [H275Y] strain of influenza virus is $T > EC_{50}$.

We analyzed these results quantitatively by examining the area under the viral burden time curves (AUC) from time zero through hour 120 and assessing the variability of the AUCs using an Analysis Of VAriance (ANOVA) with Dose as a blocking Factor and Schedule as a covariate. There is a significant dose response ($p < 0.001$) and there is a significant impact of schedule of administration on the ability to suppress viral growth over 120 hours ($p < 0.001$), with twice daily dosing being superior to daily dosing. These mathematical results further demonstrate that, for zanamivir, $T > EC_{50}$ is the dynamically-linked variable.

**Analysis of zanamivir concentrations in the HFIM system.** The data in Figure 4 show the results of the LC/MS/MS analysis of the drug concentrations in each hollow fiber unit for zanamivir. The results show that the correct concentrations of zanamivir were present throughout the first 48 hr of the experiment, indicating that the appropriate concentration time profiles were achieved in our studies.
Discussion

In this study, we examined the efficacy of the neuraminidase inhibitor, zanamivir, against wild type (A/Mexico) and oseltamivir-resistant (A/Hong Kong [H275Y]) pH1N1 influenza virus strains in the HFIM system. Although, the H275Y mutation in the neuraminidase gene of influenza confers resistance to oseltamivir, this mutation does not affect viral susceptibility to zanamivir, as illustrated in Table 1. Thus, zanamivir was chosen for study in these experiments. The goal of our experiments was two-fold: 1) to determine if zanamivir is an effective intervention against an oseltamivir-resistant influenza virus in the HFIM system; and 2) to identify optimal dosing regimens of zanamivir for oseltamivir-resistant viruses that would inhibit viral replication. As part of this, we identified the pharmacodynamically-linked index for this compound. It is important to note that clinically zanamivir is administered via two different routes: intravenous and inhalation. The experiments described in this report only focus on intravenous administration, as this is the route that will most likely be utilized for hospitalized patients with oseltamivir-resistant complicated influenza.

Our results suggest that human concentration-time profiles of the clinically used regimen of intravenous zanamivir (600 mg Q12h) were effective against the wild type pH1N1 influenza virus, A/Mexico. Zanamivir inhibited viral replication compared to the control throughout the course of the study and this inhibition was most evident at 24 hrs. Additionally, peak viral burden of the treatment arm was approximately 10-fold below that of the no-treatment control arm. These results were expected and indicate that the HFIM system is a valid method for studying dosing strategies of zanamivir against pH1N1 influenza viruses.

It is unlikely that a patient infected with a wild-type influenza virus would be treated with intravenous zanamivir, since these viruses are fully susceptible to oseltamivir. Therefore, the
main objective of our experiments was to determine if intravenous zanamivir is an effective
treatment for oseltamivir-resistant infections. To satisfy this objective, we employed the HFIM
system to perform dose range and dose fractionation studies with zanamivir against the
oseltamivir-resistant pH1N1 influenza virus strain, A/Hong Kong [H275Y]. The results of these
studies show that zanamivir, regardless of dose and schedule of administration, suppresses viral
replication relative to the no-treatment control and that the degree of suppression is dependent on
dose. It is important to recognize that there was a clear-cut exposure response by dose. Of equal
or greater significance, the schedule of administration had a significant impact upon viral
suppression, with twice-daily administration performing significantly better than daily
administration when the daily dose was matched (i.e. 1200 mg daily vs. 600 mg Q12h; 600 mg
daily vs 300 mg Q12h; 300 mg daily vs 150 mg Q12h). The dose response and the effect of
schedule were both highly significant (p << 0.001) in an analysis of variance. These results
suggest that zanamivir is effective for the treatment of oseltamivir-resistant influenza viral
infections. Additionally, this finding indicates that more frequent dosing of zanamivir is
required for optimal therapeutic outcome. Thus, we conclude from these results that the
pharmacodynamic index linked with inhibition of viral replication is Time>EC\textsubscript{50}. This is quite
different from oseltamivir, a drug of the same class, in which AUC/EC\textsubscript{50} is the
pharmacodynamic index linked with suppression of viral replication. This is highly likely due to
the difference in terminal half lives of the two drugs (37), with oseltamivir having an 8 hour
terminal half life and zanamivir having a 2.5 hour terminal half life. This issue is explored in
depth in a companion paper (4).

Our findings from the HFIM system, illustrated in Figures 2 and 3, indicate that the
clinical dosage regimen of intravenous zanamivir (600 mg Q12h) is slightly more effective
against the oseltamivir-resistant A/Hong Kong [H275Y] strain when compared to the oseltamivir-sensitive A/Mexico influenza strain. It is unlikely that this slight difference in efficacy is attributed to variations in susceptibility, since the EC$_{50}$ values for zanamivir against both viruses were nearly identical (Table 1). Differences were observed in the replication kinetics for these two viruses in the HFIM system, as the A/Hong Kong [H275Y] strain replicated faster and to higher titers in the HFIM system than the A/Mexico strain. Therefore, these relatively minor differences in zanamivir activity are most likely due to the disparity in replication rates between these two viral strains.

In order for treatment to be effective against influenza virus, zanamivir must penetrate into the lung (the site of viral replication in vivo). In our experimental design, drug was directly infused into the HFIM system to simulate the mean human pharmacokinetic profiles in serum, and not the lung. According to the zanamivir Investigator’s Brochure, lung penetration following an intravenous administration of zanamivir is 65% (56). Therefore, based on our results (Figure 3), the most conservative estimate is that the antiviral response for the drug exposures in the lung would be one-third of the way between 600 mg Q12h and 300 mg Q12h. These differences are relatively trivial and are unlikely to affect our experimental conclusions.

The results from our experiments in the HFIM system suggest that the current clinical dosing regimen of intravenous zanamivir (600 mg Q12h) is sufficient to treat patients with oseltamivir-resistant influenza infections, at least those strains harboring the H275Y single mutation, confirming the current clinical practice. Luckily, the vast majority of circulating influenza viruses is sensitive to zanamivir, but low levels of zanamivir-resistant viruses have been identified in humans throughout the world (26). Infrequent use of zanamivir is the likely explanation for the low level detection of resistant mutants in the clinic, as intravenous zanamivir
is currently only administered on a compassionate-use basis to treat severe influenza infections that are non-responsive to oseltamivir (15, 23). Based on the theory that oseltamivir-resistant viruses arose with increased oseltamivir use (42), it is quite plausible to assume that more frequent utilization of zanamivir would result in increased incidence of zanamivir-resistant influenza infections. Thus, it is imperative to identify dosing regimens that will inhibit viral replication and reduce the likelihood for the emergence of resistance. We are currently conducting studies to determine the pharmacodynamic parameter for zanamivir that is linked with suppression of the emergence of resistant viruses.

The rapid emergence and spread of drug-resistant influenza viruses highlights the need for new effective antiviral compounds. Several experimental compounds are under development for the treatment of influenza virus infections. These include the neuraminidase inhibitors A315675 (29, 41), Laninamivir (31, 51), the polymerase inhibitor, T-705 (18, 47), and the attachment inhibitor, DAS 181 (25, 36). In order for these new compounds to be effective, optimal dosing strategies must be identified.

Unfortunately, due to the high mutation rate of the virus resulting from error-prone replication, it is likely that chemotherapy with a single agent will not be adequate to treat complicated influenza infections in the near future without running the risk of resistance emergence to the single agent. Combination therapy involving two or more of these compounds will be required to cure patients with serious, life-threatening disease caused by influenza virus and markedly reduce the likelihood of resistance emergence. Several studies have begun to address this approach for the treatment of influenza infections (44, 45, 49).
In summary, we have shown that intravenous zanamivir at the clinically recommended dose is an effective treatment option for patients with complicated oseltamivir-resistant influenza infections. We have also demonstrated that the pharmacodynamic driver of this compound is Time\(> \text{EC}_{50}\) indicating that more frequent dosing intervals are required for a successful therapeutic outcome, an observation that is vastly different from other compounds in the same drug class. To our knowledge, this is the first report to definitively describe the pharmacodynamically-linked variable for zanamivir. This information plays an essential role in the design of effective dosing regimens with zanamivir against influenza viruses. Future studies will focus on using the HFIM system to identify the pharmacodynamically-linked index for resistance suppression and the exposure target required for resistance suppression and to determine optimal dosing strategies using combinations of antiviral compounds active against influenza viruses.

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Cyclopentane Neuraminidase Inhibitors with Potent In Vitro Anti-Influenza Virus

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octanoate (CS-8958) versus oseltamivir as treatment for children with influenza virus


Table 1. Antiviral activities of neuraminidase inhibitors on pH1N1 Influenza virus isolates as determined by the NA-Star® neuraminidase inhibitor resistance detection kit.

<table>
<thead>
<tr>
<th>Virus</th>
<th>Oseltamivir&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Peramivir&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Zanamivir&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; (ng/ml)</td>
<td>Standard Deviation&lt;sup&gt;c&lt;/sup&gt;</td>
<td>EC&lt;sub&gt;50&lt;/sub&gt; (ng/ml)</td>
</tr>
<tr>
<td>A/California/04/2009</td>
<td>0.072</td>
<td>0.03</td>
<td>0.018</td>
</tr>
<tr>
<td>A/New York/18/2009</td>
<td>0.081</td>
<td>0.04</td>
<td>0.030</td>
</tr>
<tr>
<td>A/Mexico/4108/2009</td>
<td>0.073</td>
<td>0.03</td>
<td>0.062</td>
</tr>
<tr>
<td>A/Hong Kong/2369/2009&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.450</td>
<td>5.44</td>
<td>3.065</td>
</tr>
</tbody>
</table>

<sup>a</sup> Virus contains the H275Y oseltamivir-resistance mutation.<br>
<sup>b</sup> The molecular weight of Oseltamivir is 284.3 g/mol, Peramivir is 328.4 g/mol, and Zanamivir is 332.3 g/mol.<br>
<sup>c</sup> Mean EC<sub>50</sub> and standard deviation values from three independent experiments.
Figure 1. Growth kinetics of pH1N1 A/Hong Kong [H275Y] (oseltamivir resistant) influenza virus and pH1N1 A/Mexico (wild type) influenza virus in the HFIM system. Each data point represents the mean viral titer between two samples and error bars correspond to one standard deviation. Similar results were obtained in two independent experiments.
Figure 2. The antiviral effect of zanamivir, given as a 600 mg dose twice daily (Q12h), on the replication kinetics of pH1N1 A/Mexico (wild type) influenza virus in MDCK cells in the HFIM system. Error bars represent one standard deviation and each data point is the mean between two samples.
Figure 3. The antiviral effect of dose and administration schedule of zanamivir on the replication of pH1N1 A/Hong Kong [H275Y] influenza virus. Each data point represents the mean of two samples and error bars correspond to one standard deviation.
Figure 4. Zanamivir concentrations in the hollow fibers units as determined by LC/MS/MS for the dose range and dose fractionation study with A/Hong Kong [H275Y]. The lines represent the targeted concentration time profiles and the triangles correspond to the observed zanamivir concentrations at the indicated time point.