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TITLE:
Safety and Pharmacokinetics of Intravenous Zanamivir Treatment in Hospitalized Adults With Influenza: An Open-label, Multicenter, Single-Arm, Phase II Study

ABSTRACT:
Background. Intravenous zanamivir is a neuraminidase inhibitor suitable for treatment of hospitalized patients with severe influenza. Methods. Patients were treated with intravenous zanamivir 600 mg twice daily, adjusted for renal impairment, for up to 10 days. Primary outcomes included adverse events (AEs), and clinical/laboratory parameters. Pharmacokinetics, viral load, and disease course were also assessed. Results. One hundred thirty patients received intravenous zanamivir (median, 5 days; range, 1–11) a median of 4.5 days (range, 1–7) after onset of influenza; 83% required intensive care. The most common influenza type/subtype was A/H1N1pdm09 (71%). AEs and serious AEs were reported in 85% and 34% of patients, respectively; serious AEs included bacterial pulmonary infections (8%), respiratory failure (7%), sepsis or septic shock (5%), and cardiogenic shock (5%). No drug-related trends in safety parameters were identified. Protocol-defined liver events were observed in 13% of patients. The 14- and 28-day all-cause mortality rates were 13% and 17%. No fatalities were considered zanamivir related. Pharmacokinetic data showed dose adjustments for renal impairment yielded similar zanamivir exposures. Ninety-three patients, positive at baseline for influenza by quantitative polymerase chain reaction, showed a median decrease in viral load of 1.42 log(10) copies/mL after 2 days of treatment. Conclusions. Safety, pharmacokinetic and clinical outcome data support further investigation of intravenous zanamivir. Clinical Trials Registration NCT01014988.

Study Design ::: METHODS:
This open-label, multicenter, single-arm, phase II study (Clinical Trials registration NCT01014988; GSK NAI113678) was conducted in 8 countries (Australia, Canada, France, Russia, Spain, Thailand, United Kingdom, and United States). The study was performed in accordance with ICH GCP and the principles of the Declaration of Helsinki, and approved by local ethics committees. Informed consent was obtained from the patient or legal guardian before the study. Enrollment criteria included age ≥18 years, hospitalization with severe or progressive laboratory-confirmed influenza [12, 13] while receiving approved influenza antiviral medications or not being suitable for treatment with approved antivirals (eg, unable to receive oral or inhaled medication), and ability to receive the first dose of intravenous zanamivir within 7 days after onset of influenza symptoms. Patients were excluded if they required concurrent therapy with another influenza antiviral medication or had elevated alanine aminotransferase (ALT) ≥3 times the upper limit of normal (ULN) and total bilirubin ≥2 × ULN, ALT >5 × ULN, or unstable cardiac disease or arrhythmia at baseline (detailed criteria available at www.clinicaltrials.gov).

Treatment ::: METHODS:
Intravenous zanamivir (600 mg) was administered over a 30-minute period twice daily for 5 days, with the option to extend treatment for up to 5 more days at the investigator's discretion. Intravenous zanamivir dosing was adjusted for patients with renal impairment based on daily calculated creatinine clearance (CLcr) or on estimated clearance while receiving continuous renal replacement therapy (CLCRRT) [14, 15] after an initial 600-mg loading dose. The maintenance dose was 600 mg for subjects with a CLcr/CLCRRT ≥80 mL/min, reduced as follows for those with lower CLcr/CLCRRT values: 400 mg for 50 to <80 mL/min, 250 mg for 30 to <50 mL/min, 150 mg for 15 to <30 mL/min, and 60 mg for <15 mL/min. The interval between the initial dose and the start of maintenance dosing was 24 hours for patients with a CLcr/CLCRRT of 15 to <30 mL/min and 48 hours for those with a CLcr/CLCRRT of <15 mL/min. For all other patients, maintenance doses were administered every 12 hours (Supplementary Table 1). The dose rationale was based on the prophylactic efficacy of intravenous zanamivir [16] concentrations in lung epithelial lining fluid (range, 216–1163 ng/mL) were many times greater than the median inhibitory concentration for a range of influenza A and B neuraminidases after intravenous administration of zanamivir (600 mg); these concentrations were 55%–79% of the corresponding serum zanamivir concentrations [17].

Outcome Measures ::: METHODS:

Primary safety outcomes included adverse events (AEs) classified according to the DAIDS toxicity scale [18], serious AEs (SAEs), incidents of hepatic injury (liver AEs defined as ALT ≥5 × ULN; liver SAEs defined as ALT ≥3 × ULN and total bilirubin ≥2 × ULN; laboratory criteria of Hy's law [19]), clinical laboratory measurements, electrocardiographic data, and vital signs.
Secondary outcome measures included serum pharmacokinetic parameters and change in influenza viral load over time (by quantitative real-time polymerase chain reaction [qRT-PCR] and quantitative virus culture [qVC]; Quest Diagnostics). Clinical end points included mortality rate, length of hospitalization (as measured from study day 1), intensive care unit (ICU) stay (total length of stay), and time until return to normal vital signs, according to defined criteria. Exploratory outcomes included influenza viral load quantification in samples obtained from endotracheal aspirates and correlation analyses between pharmacokinetic parameters, viral load, and clinical outcomes.

Study Procedures ::: METHODS:
Safety and clinical outcomes were assessed daily during treatment, then after treatment on days 2, 5, 9, 16, and 23 after the last dose of intravenous zanamivir. Poststudy deaths attributable to AEs that began during the study (or occurring during the period of hospitalization) were also recorded, even if death occurred after the end-of-study assessment.
Serum pharmacokinetic sampling was optional. Pharmacokinetic samples for the initial dose were scheduled for collection before and at 25–30 minutes (end of infusion) and 1–2, 4–6, and 11–12 hours after the start of infusion. If the start of maintenance dosing was delayed for renal impairment, then additional samples were scheduled for 22–24 and 46–48 hours after the dose. For the maintenance dose on day 3, 4 or 5, sample collections were as for day 1, up to 12 hours after the dose.
Serum zanamivir concentrations were measured using a validated assay based on protein precipitation, followed by high-performance liquid chromatography–tandem mass spectrometry analysis. For a 50-μL aliquot of serum, the lower limit of quantification (LLQ) was 10 ng/mL, and the upper limit 10 000 ng/mL. Pharmacokinetic parameters were estimated from concentration-time data by standard noncompartmental analysis using WinNonlin software, professional version 5.2 (Pharsight).
Nasopharyngeal swab samples (Copan Diagnostics) to assess viral load and influenza subtype were collected on days 1–5 of treatment. Additional samples were taken on days 7 and 10 if dosing was continued beyond day 5, and on posttreatment assessment days if patients continued to be hospitalized and symptomatic. Optional endotracheal samples were collected at a single time point between days 3 and 5. RNA was isolated from nasopharyngeal and endotracheal samples, and 1-step qRT-PCR was used to quantify levels of influenza RNA. The LLQ for the assay was set at 2.7 log10 (500) copies/mL. Viral titers were also deduced by median tissue culture infectious dose (TCID50) calculation after serial dilution of samples, followed by adsorption onto Madin-Darby canine kidney cells. The number of plaque-forming units was used to calculate the TCID50, with an LLQ of 0.4 log10 TCID50/mL.

Statistical Analysis ::: METHODS:
The planned sample size for the study was chosen to provide enough patients to determine the safety and tolerability of intravenous zanamivir in the patient population. With 130 enrolled patients, we can exclude AEs with a frequency >2.8% with 95% confidence. Exploratory analyses of Cox regression and Pearson correlation were performed to investigate associations between various outcomes, such as mortality, clinical and virologic responses, hospital or ICU stay, clinical risk factors, and pharmacokinetic parameters. Wilcoxon signed rank test was performed to compare viral loads between endotracheal and nasopharyngeal samples.

Study Population ::: RESULTS:
Between November 2009 and September 2011, 130 adult patients were enrolled from 30 centers. Three patients were pregnant (1 second trimester, 2 third trimester), and 1 patient was 1 day post partum. Patients were treated for a median of 5 days (range, 1–11 days): 87 patients (67%) received intravenous zanamivir for ≤5 days, and 43 (33%) received intravenous zanamivir for >5 days. Thirty patients (23%) were prematurely withdrawn from intravenous zanamivir treatment, 12 (9%) owing to (on-treatment) fatality, 11 (8%) at the discretion of the investigator (most because of clinical improvement and hospital discharge), 4 (3%) owing to AEs (cytolytic hepatitis, hepatic enzyme elevation, renal failure, and rash), 2 (2%) owing to withdrawal of consent, and 1 owing to

protocol deviation. Twenty-three patients (18%) did not complete the study; 20 (15%) owing to death, and 1 each owing to withdrawal of consent, investigator discretion, or loss to follow-up. Baseline patient characteristics and demographics are summarized in Table 1. The most common influenza symptoms at baseline were fever (82%), cough (81%), and dyspnea (72%); 77% of patients had ≥1 chronic underlying medical condition (Table 1). Ten patients (8%) had documentation of influenza vaccination in the 9 months before presentation. Chest radiographic evidence of pneumonia or pneumonitis was present at the baseline in 112 of 126 patients (89%). The median time from symptom onset to initiation of intravenous zanamivir was 4.5 days (range, 1–7 days), and 104 patients (80%) received oseltamivir before study entry (median exposure, 2 days). The most common influenza type/subtype was A/H1N1pdm09 (71%), followed by A/H3N2 (12%), influenza A subtype unknown (11%), and influenza B (2%). Four percent of isolates could not be typed.

Safety End Points ::: RESULTS:
Overall AEs, SAEs, and grade 3 or 4 AEs were reported in 110 (85%), 44 (34%), and 57 (44%) patients, respectively. Summaries of SAEs and grade 3/4 AEs are presented in Table 2 and Table 3. A summary of all AEs is presented in Supplementary Table 2.

In total, 28 patients (22%) reported AEs considered by the investigator to have a possible causal relationship to zanamivir, the most common were acute liver injury in 13 patients (10%), rash in 4 (3%), and thrombophlebitis or venous thrombosis in 4 (3%). SAEs considered by the investigator to be possibly zanamivir related included 2 cases of ventricular arrhythmia, 2 of acute liver injury meeting laboratory criteria of Hy's law, 2 of encephalopathy, and 1 of renal failure. One event of ventricular arrhythmia (torsade de pointes) reported as possibly related to intravenous zanamivir occurred 16 days after completion of zanamivir treatment, and was confounded by haloperidol treatment.
Seventeen patients (13%) experienced protocol-defined liver AEs (n = 14; 11%) or SAEs (n = 3; 2%). The median time from initiation of intravenous zanamivir to onset of liver AEs was 9 days (range, 1–27 days), and the median time from the last dose of intravenous zanamivir to the onset of liver AEs was 1 day (range, 1–22 days). Except for 1 patient who died of an unrelated cause on day 3, all liver SAEs and AEs resolved or improved by the end of follow-up (about 3 weeks after the last dose of intravenous zanamivir). Eleven AEs and 2 SAEs were considered by the investigator to be potentially attributable to intravenous zanamivir. Of the 3 patients with liver SAEs (ALT ≥3 × ULN and total bilirubin ≥2 × ULN), 1 experienced an SAE that resolved by the end of the study, 1 died of cardiogenic shock (unrelated to intravenous zanamivir), and 1 (with confounding hepatitis C and a liver event not attributable to study drug) died of hemothorax and multiorgan failure. Most protocol-defined liver events were associated with A/H1N1pdm09 infection and multiorgan failure. In the overall study population, there were no changes in median ALT, aspartate aminotransferase, or total bilirubin levels during or after treatment.
Twenty-six patients died, for an overall cumulative mortality (including poststudy deaths) of 20%; 14- and 28-day cumulative mortality were 13% (n = 17) and 17% (n = 22), respectively. The most common causes of death were respiratory failure (n = 7; 5%), sepsis or septic shock (n = 5; 4%), cardiogenic shock (n = 4; 3%), and bacterial pulmonary infections, including pneumonia and bronchopneumonia (n = 4; 3%). None of the deaths was considered by the investigator to be attributable to zanamivir treatment. No other safety signals or clinically significant trends in laboratory values, vital signs or electrocardiographic findings were identified or considered attributable to zanamivir. All 3 pregnant patients survived and gave birth to healthy infants. No SAEs were reported in the pregnant or postpartum patients.

Serum Pharmacokinetics ::: RESULTS:
Serum samples were obtained in 126 (97%) patients for pharmacokinetic analysis. Results are provided in Table 4 and Table 5. For the initial 600-mg dose on day 1 (Table 4), area under the serum concentration–time curve extrapolated to infinity (AUC(0-∞)) values typically increased with decreasing CLcr, from 82.9 h · μg/mL for patients with CLcr ≥80 mL/min to 950 h · μg/mL for patients with CLcr <15 mL/min No differences were observed in maximum plasma concentration (Cmax) between the renal function groups (range of group means, 32.8–47.1 μg/mL). During maintenance dosing, as expected, the maximum plasma concentration decreased and the trough

plasma concentration (Cmin) increased with reduced doses for renal impairment, but similar AUCs were observed (Table 5).

Virology ::: RESULTS:
In 93 of 124 patients (75%) with influenza qRT-PCR–positive nasopharyngeal samples at baseline, the median viral load was 5.34 log10 copies/mL, which decreased at day 3 (2 days of treatment) by 1.42 log10 copies/mL (Figure 1). The median time to no detectable virus RNA by qRT-PCR was 3 days (range, 1–31; interquartile range, 1–5). Only 54 of 126 patients (43%) had positive qVC results from nasopharyngeal samples at baseline; thus, the median qVC result was below the LLQ. We found that qVC results in this study were less reliable than qRT-PCR results and were not used for analyses. We identified no baseline or emergent H275Y, I223R/V/K, or Q136K mutations. Detailed genotypic, phenotypic, and minority species analyses will be reported in a separate publication.

Twenty-two of 23 patients had an endotracheal sample positive for influenza by qRT-PCR (influenza A/H1N1pdm09 in 19, A/no subtype in 2, and A/H3N2 in 1). The median viral load was 4.68 log10 copies/mL. A total of 21 patients had paired qRT-PCR data in both endotracheal and nasopharyngeal samples, with median viral loads of 4.89 and 3.60 log10 copies/mL, respectively (P = .004); of these, 17 (81%) had higher viral loads in endotracheal samples (Supplementary Figure 1; Supplementary Appendix).

Clinical End Points ::: RESULTS:
The median duration of hospitalization was 15 days (range 1–133 days). One hundred eight patients (83%) had an ICU stay during the study; the median duration was 11.5 days (range, 1–104 days). The median time to return to predefined normal criteria for each vital sign was between 2 and 8 days, but data were highly variable (Table 6).

Sixty-four patients (49%) received systemic corticosteroids while receiving treatment with intravenous zanamivir. The median time to a qRT-PCR result <500 copies/mL was 3 days among patients who received corticosteroids (range, 1–31 days), compared with 2 days among those who did not (range, 1–27 days). The cumulative mortality rate was 22% (14 of 64 patients) among patients who received corticosteroids compared with 18% (12 of 66) among those who did not. The overall rate of infection-related SAEs was lower in the corticosteroid group (11% vs 18%), but 3 fatal cases of pulmonary aspergillosis occurred, all among patients who received corticosteroids.

Exploratory Analyses ::: RESULTS:
The effect of antiviral treatment on mortality rates was explored using Cox modeling. Time-dependent exposure to intravenous zanamivir was associated with an adjusted hazard ratio (aHR) of 0.793 (95% confidence interval [CI], .230–2.736), accounting for prior or subsequent oseltamivir exposure (aHR, 0.731; 95% CI, .092–5.827) and other potential risk factors for increased mortality rates (Table 7). The univariate analysis showed that both H3N2 subtype and age were significantly associated with mortality rates, but corticosteroid use was not (hazard ratio, 1.17; 95% CI, .54–2.53). There was no correlation between higher zanamivir exposure and drug-related AEs or protocol-defined liver events. A weak but significant correlation (Pearson R = 0.28; P = .003) was noted between AUC(0-∞) and the occurrence of SAEs, but this was confounded by the presence of renal dysfunction at baseline. We found a weak correlation between AUC(0-∞) and decrease of nasopharyngeal qRT-PCR on day 3 of treatment (Pearson R = –0.23; P = .02).

We explored whether there was a relationship between baseline levels of influenza RNA by qRT-PCR and mortality rate by grouping patients with positive baseline qRT-PCR results (93 patients) in qRT-PCR tertiles and comparing them with those who had negative qRT-PCR resutls at baseline (31 patients; 6 had missing values at baseline). Patients in the 2 highest tertiles of influenza qRT-PCR had cumulative mortality rates of 23% and 22%, respectively; patients in the lower tertile (<4.57 log10 copies/mL) had a cumulative mortality rate of 17%, and this rate among patients with negative baseline qRT-PCR results was 13%. We also explored whether the change in nasopharyngeal influenza viral load from baseline to day 3 (n = 82 with paired samples) was associated with mortality rates. We found no association between change in viral load from

baseline and mortality rate by Cox modeling (P = .92); deaths were evenly distributed between those who experienced changes from baseline greater than −1.42 log10 by treatment day 3 (8 subjects) and those who experienced smaller changes (7 subjects).

DISCUSSION:

In this open-label, international, phase 2 trial we prospectively assessed the safety, tolerability, virologic effects, and pharmacokinetics of intravenous zanamivir (600 mg every 12 hours, adjusted for renal dysfunction) in 130 hospitalized adults with confirmed influenza, most of whom had associated pneumonia and were treated in an ICU. The all-cause cumulative mortality rate in this trial was 20%, and the 28-day mortality rate was 17%. Both intravenous zanamivir and oseltamivir use were associated with lower aHR for death when modeled as time-dependent exposures. The observed mortality rate was lower but consistent with retrospective cohort studies of critically ill hospitalized patients with influenza during this time period [20–25]. For example, in critically ill patients with 2009 influenza A/H1N1pdm09, a 50% ICU mortality rate has been reported [25], and a California-cohort study in 1950 patients reported a 25% mortality rate with neuraminidase treatment (median time from symptom onset, 4 days) and a 42% mortality rate without treatment [26]. Of the 26 deaths in this study, none was thought by the treating physician to be attributable to zanamivir. The small number of pregnant women did well clinically, with no observed adverse fetal effects.

Influenza viral load analyses suggested that intravenous zanamivir had a rapid antiviral effect, with a median decrease in viral load of 1.42 log10 copies/mL after 2 days of treatment, despite the presence of symptoms for a median of 4.5 days before study entry and prior use of oseltamivir in 80% of patients. This viral load reduction is consistent with findings of previous studies of inhaled zanamivir in acute uncomplicated seasonal influenza and limited data from hospitalized patients during the pandemic [27, 28]. In a Hong Kong study of 66 adults with influenza A/H1N1pdm09, patients with severe pneumonia experienced a longer duration of viral RNA positivity (by nasopharyngeal swab sample) after starting oseltamivir treatment (median, 6 days; range, 3–8) than those with milder illness (median, 2 days; range, 1–3) [29]. In our study, higher virus loads were observed in endotracheal samples than in simultaneous nasopharyngeal samples, reflecting the lower respiratory tract burden of influenza illness in this patient population [30]. In this study, we found a poor correlaton between the qVC and qRT-PCR results. The reason is not clear, but the qRT-PCR data were considered more reliable and have been presented to describe antiviral efficacy.

In most cases, the causal connection between AEs and zanamivir was confounded by influenza severity, underlying medical conditions, and numerous concomitant medications, including antibiotics in 92% (data not shown). Protocol-defined liver events were observed in 13% of the study population; in all patients except 1 who died imminently of cardiogenic shock, liver enzyme elevations resolved with resolution of influenza or critical illness. Intravenous zanamivir was not noted to increase liver chemistry values in phase I healthy volunteer studies or preclinical animal safety studies [31, 32]. In our study, liver events did not correlate with higher zanamivir exposure, and there were no overall increases or discernible pattern in liver enzyme results, with many patients experiencing liver enzyme elevations at baseline, during treatment, or after treatment. Liver enzyme elevation has also been described in critically ill patients with influenza A/H1N1pdm09 [33]. It is unclear whether intravenous zanamivir had a role in causing or exacerbating underlying liver inflammation. To address this uncertainty further, a current phase 3 trial of intravenous zanamivir in hospitalized adults with influenza is testing 2 dose levels of intravenous zanamivir (600 or 300 mg twice daily) and comparing them with oral oseltamivir (clinicaltrials.gov, NCT01231620).

Zanamivir pharmacokinetic parameters in this severely ill population were generally consistent with results of previous studies in healthy volunteers [34], although variability was greater. Dose adjustments for patients with renal impairment resulted in AUCs similar to those in patients with normal renal function. Pharmacokinetic parameters for patients receiving continuous renal replacement therapy or extracorporeal membrane oxygenation seemed similar to those for patients not undergoing these procedures (data not shown). However, few patients underwent continuous renal replacement therapy (n = 14) or extracorporeal membrane oxygenation (n = 4), and only 4 had simultaneous pharmacokinetic parameters. Thus, additional data are required to confirm these findings.

This study was limited by its single-arm, uncontrolled, open-label design and the critically ill nature of the patient population. Although a potential clinical benefit is difficult to assess without a

control group, the safety and clinical outcomes observed in this study are consistent with those expected in patients with severe influenza and reflect a real-life clinical setting.