Evaluation of the Antiviral Response to Zanamivir Administered Intravenously for Treatment of Critically Ill Patients With Pandemic Influenza A (H1N1) Infection

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A retrospective nationwide study on the use of intravenous (IV) zanamivir in patients receiving intensive care who were pretreated with oseltamivir in the Netherlands was performed. In 6 of 13 patients with a sustained reduction of the viral load, the median time to start IV zanamivir was 9 days (range, 4–11 days) compared with 14 days (range, 6–21 days) in 7 patients without viral load reduction ($P = .052$). Viral load response did not influence mortality. We conclude that IV zanamivir as late add-on therapy has limited effectiveness. The effect of an immediate start with IV zanamivir monotherapy or in combination with other drugs need to be evaluated.

During last year's influenza pandemic, life-threatening disease was encountered in a proportion of patients [1–4]. Despite extensive treatment, the mortality rate among these patients remained high [2]. One of the problems encountered was the development of resistance to oseltamivir. In addition, in isolated cases, oseltamivir use was complicated due to absent gastric mobility [5]. Currently, zanamivir is the alternative drug of choice to use when oseltamivir-resistant viruses develop. However, at this moment it is only approved for use as an inhaled formulation. Respiratory problems following inhalation, dysfunction of ventilators due to blockade of the filters by the lactose carrier, and suboptimal drug disposition are issues associated with its formulation [6, 7]. The use of intravenous (IV) zanamivir may be an option to overcome these problems. Currently, only limited peer-reviewed data in humans and one study in primates have been published [8–12]. We here report a retrospective observational nationwide study in the Netherlands on the antiviral efficacy of IV zanamivir.

METHODS

Patients included in this study were identified, retrospectively, through a request to all registered medical microbiologists in the Netherlands. A standardized form was provided to collect relevant clinical and virological data. All patient data were reported anonymously. The decision to start IV zanamivir and the duration of therapy were not standardized and depended on the clinical judgment of the treating physician. Initially, compassionate use was granted by GlaxoSmithKline; from January 2010 onward, this was continued by the National Institute for Public Health and the Environment. Intravenous zanamivir was provided for patients unable to use oseltamivir or in whom treatment with IV zanamivir was considered desirable for other reasons. Nasopharyngeal swabs, nose washes (upper respiratory tract [URT] samples), tracheal aspirates, and broncheo-alveolar lavages (lower respiratory tract [LRT] samples) were collected during routine care.

Inclusion criteria for patients in this study were as follows: laboratory-confirmed pandemic influenza A (H1N1) infection by real-time reverse-transcription polymerase chain reaction (RT-PCR); admission to an intensive care unit (ICU) in a Dutch hospital; use of IV zanamivir for >48 hours; and availability of follow-up virological samples. Patients with proven resistance to

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zanamivir at baseline or at ICU admittance were excluded from the study.

Detection and quantification of viral RNA and screening for the neuraminidase (NA) H275Y oseltamivir resistance mutation was performed in all centers by means of standardized RT-PCR–based methods. To obtain sequences of NA genes, viral RNA was reverse transcribed and subsequently amplified using gene-specific primers (sequences are available upon request). Attempts to culture virus were made at sample viral loads of $>4 \times 10^3$ virus particles (vp) per milliliter. Phenotypic sensitivity to oseltamivir and zanamivir was measured using the NA-star neuraminidase inhibitor resistance detection kit (Applied Biosystems, Nieuwerkerk aan den IJsel, the Netherlands). See the supplementary data for more technical details.

Clinical data were extracted from the medical records. Immunosuppression was defined as any of the following: receipt of treatment for any cancer within 6 months before influenza infection, the use of any immunosuppressive medication to prevent transplant rejection or for management of pulmonary or autoimmune conditions, or a diagnosis of AIDS. Acquired respiratory distress syndrome (ARDS) was defined as bilateral infiltrates on chest radiographs, with a Pa_{O_2}/F_{IO_2} ratio of <200 mm Hg in the absence of cardiogenic pulmonary edema.

The viral response to treatment with IV zanamivir was determined by the differences in viral load between a sample collected closest to the day of the start of IV zanamivir (baseline sample) and those obtained at follow-up. Sustained reduction of the viral load was defined as a decrease of $\geq 1 \log_{10}$ vp/mL for at least 10 days. In case of death or cessation of IV zanamivir treatment, the last sample collected while the patient was still receiving therapy was considered for the endpoint of analysis. If the reduction of the viral load took >7 days of treatment to occur, it was considered not to be treatment related. For statistical analysis between groups, the Mann–Whitney *U* test was used. *P* values of <.05 were considered to be significant.

The study protocol was reviewed and approved by the medical ethics board of the Erasmus University Medical Center (study no. 2010-162). Informed consent was waived because patient inclusion was performed retrospectively and anonymously. There was no sponsor involvement in the design, data collection, and analysis of this study.

RESULTS

Based on data provided by GlaxoSmithKline during the influenza season in the Netherlands, 26 ICU patients were treated with IV zanamivir. Overall, we obtained clinical data of 19 patients from 6 hospitals. Of these 19 patients, 6 did not meet the inclusion criteria. Reasons for exclusion from the study were death ($n = 2$), recovery and treatment interruption within 48 hours after start of IV zanamivir ($n = 2$), absence of samples for virological evaluation ($n = 1$), and proven resistance to zanamivir before admittance to the ICU ($n = 1$).

The patient characteristics are summarized in Table 1. Most patients had a pre-existing medical condition and were immunocompromised. All patients were pretreated with oral oseltamivir for a median period of 5 days (range, 1–18 days). Oseltamivir was dosed at 75 mg every 12 hours or its pediatric equivalent, except in 3 patients (patients 4, 5, and 8) in whom 150 mg every 12 hours was used. Three patients received IV zanamivir monotherapy (patients 7, 9, and 13), whereas the remaining 10 patients received IV zanamivir combined with continued use of oseltamivir. No other antiviral therapy was prescribed.

The median baseline viral load was 3×10^4 vp/mL, (range, <17–1.2×10^8 vp/mL) in the URT samples and 2.3×10^5 vp/mL (range, <17–9.8×10^6 vp/mL) in the LRT samples. Two patients (patients 1 and 12) had an undetectable viral load in the URT sample but a high viral load in the LRT sample at baseline. One patient (patient 5) had an undetectable viral load in the LRT sample but a high viral load in the URT sample at baseline. The response analysis was complicated for 2 patients because their baseline samples were obtained 3 days before IV zanamivir initiation (patients 8 and 10). Overall, sustained reduction of the viral load was observed in only 6 patients (Figure 1). Three patients had persistent undetectable viral loads after 7 days of treatment (patients 7, 9, and 13). There were no significant differences between baseline viral loads in the URT and LRT samples of patients with and without viral load reduction ($P = .315$ and $P = .250$, respectively). Overall, the median interval between first onset of symptoms and start of IV zanamivir was 11 days (range, 4–21 days). However, among patients who had a sustained reduction of the viral load, the median time to start of IV zanamivir was 9 days (range, 4–11 days), compared with 14 days (range, 6–21 days) among patients in whom treatment had no effect ($P = .05$). All 3 patients receiving monotherapy with IV zanamivir showed a sustained viral load response.

A sequence could be obtained from viruses isolated from 10 patients before start of IV zanamivir, but sequences could not be obtained from viruses isolated from patients 7, 9, and 12. Viruses isolated from patients 3, 6, 7, and 13 carried the H275Y oseltamivir resistance mutation in NA as determined by sequencing and/or RT-PCR specific for the H275Y mutation. No other mutations associated with drug resistance were found in the NA genes. For 9 patients, phenotypic resistance data could be measured in isolates obtained before start of IV zanamivir. All 9 isolates were susceptible to zanamivir (Table 1).Virus could not be cultured from all 19 samples eligible for culture that were obtained after the start of IV zanamivir, despite relatively high loads with a median of 4.0×10^5 vp/mL (range, 4.9×10^3–14×10^5 vp/mL).

Twelve patients were admitted to the ICU because of respiratory failure; 1 patient was already admitted. All patients needed supplemental oxygen: 12 patients were mechanically

Table 1. Selected Patient Characteristics and Outcomes of 13 Patients Receiving IV Zanamivir

						Viral resistance to Oseltamivir		Baseline viral load, vp/mL					
Patient no.	Underlying disease	Age in years	Immunocompromised	ARDS	Oseltamivir pretreatment, days	Amino acid position 275 in the viral NA (N1 numbering)	IC_{50}, nmol/L	URT sample	LRT sample	Time to start of Zanamivir, days	Zanamivir IC_{50}, nmol/L	1 $\log_{10}$ decrease in viral load	Outcome
1	None	46	No	Yes	4	H	0.4 (S)	ND	4.9×10^3	12	0.1 (S)	No	Survived
2	Cerebral vasculitis, pulmonary embolisms long-term high-dosage prednisone use	41	Yes	Yes	14	H	NR	1.0×10^5	…	14	NR	No	Died
3	Chronic myeloid leukemia, bone marrow transplantation, graft-vs-host disease, clinical significant aortic valve stenosis	58	Yes	Yes	18[a]	H	132.8 (R)	4.0×10^4	3.76×10^5	19	0.2 (S)	No	Died
4	None	14	No	Yes	1	H	0.2 (S)	2.0×10^5	9.8×10^6	4	0.2 (S)	Yes	Died
5	Down syndrome, acute lymphoblastic leukemia	5	Yes	Yes	5	H	0.4 (S)	1.0×10^4	ND	14	0.1 (S)	No	Survived
6	Follicular lymphoma	59	Yes	Yes	7	H/Y[b]	7.1 (R)[b]	1.2×10^8	…	11	0.4 (S)	Yes	Died
7	Minimal change nephropathy, prednisone use	39	Yes	Yes	7	Y	NR	…	9.0×10^3	9	NR	Yes	Survived
8	Obesity	42	No	Yes	4	H	0.4 (S)	4.3×10^4	6.5×10^5	6	0.1 (S)	No	Survived
9	Obesity, ovarian adenoma (identified during hospital admission)	30	No	Yes	6	NR	NR	4.5×10^2	…	9	NR	Yes	Survived
10	Systemic lupus erythematosis	40	Yes	Yes	3	H	0.3 (S)	…	3.5×10^6	10	0.2 (S)	Yes	Died
11	Acute myeloid leukemia	49	Yes	Yes	6	H	0.3 (S)	3×10^4	…	10	0.3 (S)	No	Died
12	Alcohol abuse	52	No	Yes	1	NR	NR	ND	9.1×10^5	21	NR	No	Survived
13	Biliary atresia, hepatocellular carcinoma, liver transplantation	1	Yes	No	9	Y	NR	3.0×10^4	…	11	0.2 (S)	Yes	Survived

NOTE. Reference median inhibitory concentration (IC_{50}) values ($n = 60$) for oseltamivir are 0.20 ± 0.08 nmol/L (median +/− 95% CI) and for zanamivir are 0.22 ± 0.12 nmol/L. ARDS, acquired respiratory distress syndrome; H, histidine; IC_{50}, median inhibitory concentration; LRT, lower respiratory tract; NA, neuraminidase; ND, not detectable; NR, no result could be obtained; R, resistant; S, susceptible; URT, upper respiratory tract; vp, virus particles; Y, tyrosine.

[a] Plus inhaled zanamivir therapy for 9 days.

[b] Virus mixture of wild type and mutants with an H275Y mutation.

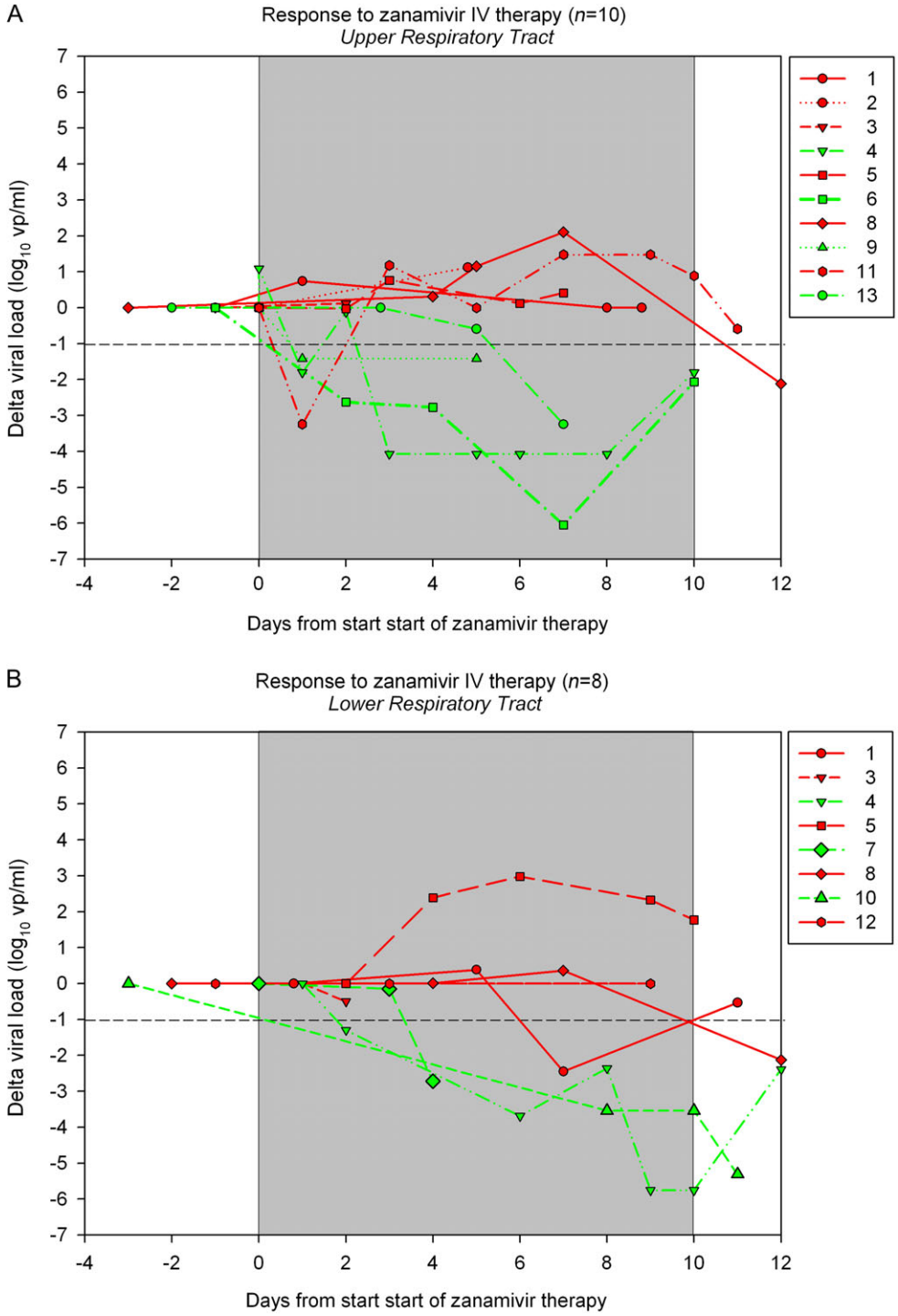

Figure 1. Virological response to intravenous (IV) zanamivir in 13 patients. *A,* Change in baseline viral load detected in the upper respiratory tract (URT); *B,* change in baseline viral load detected in the lower respiratory tract (LRT). Eight patients were treated for >10 days, and 5 patients were treated for <10 days. Of those 5 patients, 2 died and 3 clinically recovered, and zanamivir IV medication was stopped (patients 7, 9, and 13). Because patient 12 had undetectable viral loads in the URT sample, these data are not included in the figure for change in baseline URT viral load. Each line represents a single patient. Data obtained from patients with a sustained viral load reduction are depicted in green, and data for those without a viral response are depicted in red; the black dashed line represents the delta viral load of -1 $\log_{10}$ virus particles (vp) per milliliter.

ventilated and 4 patients required additional extracorporeal membrane oxygenation support. In all mechanically ventilated patients, ARDS was diagnosed. The overall mortality was significant, with a total number of 6 deaths. There was no relation between viral response and survival, with death occurring in 3 of 6 patients in the group with sustained viral load reduction

and in 3 of 7 patients in the nonresponder group. Most deaths (5 of 6) occurred in patients with an impaired immune system. The remaining 7 surviving patients were discharged after a median hospital stay of 52 days (range, 11–62 days), of which 37 days (range, 39–77 days) were spent in the ICU. All patients received broad-spectrum antibiotics at least once. In none of the patients was IV zanamivir stopped because of serious adverse events.

DISCUSSION

In this study, which is to our knowledge the largest published peer-reviewed case series to date, we have retrospectively analyzed the virological response to IV zanamivir treatment in patients pretreated with oral oseltamivir for severe influenza infection. A consistent virological response to add-on IV therapy was seen in only 6 of 13 patients. There was a trend toward an increased likelihood of response for those who started IV zanamivir at an earlier stage of disease.

This study has several limitations. Due to the retrospective nature, the absence of a control group, and the limited number of patients enrolled, we cannot make a definitive conclusion on the clinical efficacy of IV zanamivir. Still, we feel that our observations are important, because they are not in line with previously published reports on individual patients with encouraging results [8–10]. Several factors may explain the limited effectiveness of IV zanamivir seen in 7 of 13 patients. First, the patients included were severely ill as reflected by the high number of immunocompromised patients, poor response to oral oseltamivir, and extended duration of hospitalization. A potential explanation for the limited virological efficacy may have been the development of viruses resistant to zanamivir [13]. Unfortunately, because we were unable to obtain viral isolates or relevant viral sequences after start of zanamivir therapy, it remains unknown to what extent resistance contributed to the absence of virological response. Another explanation for the absence of response could be that insufficient zanamivir levels were achieved at some sites of active viral replication. Further detailed pharmacological studies are warranted to investigate this possibility. Of interest is a recent finding in patients with influenza A (H3N2) infection that the combination of oseltamivir and zanamivir may be associated with a decreased inhibition of viral replication. However, in that study all patients did clear the virus rapidly [14]. In this light, it is important to note that all patients described in our study had been pretreated with oseltamivir monotherapy. Resistance to oseltamivir was found in only 4 of them. Therefore, we hypothesize that the same mechanism accounts for the limited efficacy of both IV zanamivir and oral oseltamivir.

Because of the prolonged viral replication before the start of IV zanamivir, irreversible lung injury may already have occurred. Thus, the relatively late start with IV zanamivir in the course of disease may have compromised its potential efficacy. This is suggested by the shorter intervals between illness onset and initiation of IV zanamivir in patients with a sustained suppression of the viral load. The extensive damage to the lungs may have resulted in viral salvage sites inaccessible to the immune system and antiviral medication. Earlier start of IV zanamivir may help to prevent formation of these sites. Indeed, previously published data show that early start with neuraminidase inhibitors results in a better outcome in hospitalized patients [15]. Finally, it is well recognized that bacterial superinfections may also contribute to clinical deterioration; we cannot rule out the possibility that this has also occurred in our population, despite the fact that all of our patients received antibiotic therapy.

In conclusion, the use IV zanamivir as late add-on therapy for H1N1 virus–infected patients in the ICU showed limited antiviral effectiveness. In our selected population, reduction of viral load did not affect mortality. Further studies evaluating earlier start with IV zanamivir monotherapy therapy alone or in combination with other treatment strategies are needed.

Supplementary Data

Supplementary Data are available at *The Journal of Infectious Diseases* online.

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