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Short Article

Clinical and virologic outcomes in patients with oseltamivir-resistant seasonal influenza A (H1N1) infections: results from a clinical trial

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Nineteen patients with oseltamivir-resistant seasonal influenza A (H1N1) infections were randomized to receive oseltamivir or placebo. Nasopharyngeal swabs were obtained, and clinical and virologic outcomes were compared, stratified by early or late treatment. Neuraminidase inhibition assay and pyrosequencing for H275Y confirmed resistance. Twelve (63%) patients received oseltamivir; 8 (67%) received late treatment. Seven (37%) patients received placebo; 6 (86%) presented >48 hours after onset. Time to 50% decrease in symptom severity, complete symptom resolution, and first negative culture were shortest among the early treatment group. While sample size prohibits a strong conclusion, future studies should evaluate for similar trends.

Keywords Antiviral, influenza, oseltamivir effectiveness, resistance.

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Introduction

The Monitoring Influenza Severity and Transmission on Tamiflu (MISTT) study is a double-blind, placebo-controlled clinical effectiveness trial that began January 2008 aiming to assess the duration of clinical symptoms and viral shedding in patients treated early (≤48 hours after symptom onset) or late (>48–119 hours after symptom onset) with influenza antiviral therapy compared with placebo. On February 11, 2009, enrollment was temporarily suspended because of a high prevalence of oseltamivir-resistant seasonal influenza A (H1N1) viruses among study participants and in the community. By that time, 21 patients infected with oseltamivir-resistant A (H1N1) had been enrolled and randomized. These patients provided a unique opportunity to evaluate the effect of oseltamivir treatment on the clinical outcomes of patients infected with influenza viruses that were considered to be resistant to oseltamivir by *in vitro* antiviral resistance assays.

Methods

Outpatients seeking medical attention for acute respiratory illness were recruited from a defined population living near Marshfield, Wisconsin,[1] between January 19, 2009 and February 11, 2009. Consenting patients aged 12 months through 79 years with feverishness, chills, or cough <120 hours in duration were invited to participate after an outpatient encounter for acute respiratory illness if their health care provider did not prescribe antiviral agents. Nasal swabs (children) or nasopharyngeal swabs (adolescents and adults) were obtained from all consenting patients for rapid antigen test (Binax NOW® Influenza A & B test kit; Binax, Inc., Portland, ME, UK) and reverse transcription polymerase chain reaction (RT-PCR) to detect influenza A and B using the LightCycler® Real-Time PCR System (Roche Diagnostics, Basel, Switzerland), as previously described.[1] Patients with influenza detected by either assay were immediately randomized to receive a

5-day course of oseltamivir (standard dose for age) or placebo in a 2:1 ratio.

Each randomized participant or guardian recorded temperature and symptoms (feverishness, cough, fatigue, nasal congestion, wheezing, headache, muscle aches, and sore throat), for a minimum of 7 days or until symptom resolution, up to a maximum of 14 days. Each symptom was scored on a scale of 0 (absent) to 3 (severe), and a composite severity score was calculated as the sum of all individual symptom scores for a 12-hour reporting period. Symptom resolution was defined as occurring when all symptoms were absent (scored as 0) or mild (scored as 1) for two consecutive reporting periods (approximately 24 hours) during which no over-the-counter decongestants, antitussives, or antipyretics were used.

All participants received a follow-up swab for RT-PCR and viral culture on day 3 or 4 after randomization (day 0) and participants <18 years old were invited to enroll in a viral shedding study with serial swabs collected on days 1, 2, 4, and 6 after randomization. MDCK shell vial cell culture was performed on all specimens that were positive for influenza by RT-PCR and on all serial samples collected. Oseltamivir resistance was identified by the chemiluminescence neuraminidase inhibition assay (NI),[2] and the presence of H275Y in the neuraminidase (NA) was determined by pyrosequencing.[3]

We compared time to clinical and virologic resolution across treatment groups. For virologic outcome measures, we divided the patients into two groups: participants who provided multiple swabs after randomization (the viral shedding substudy participants) and those that provided only a single follow-up swab. Smoothed severity score profiles across time were generated for each treatment group using penalized B-splines.[4] Comparisons were conducted using the exact Wilcoxon rank-sum test. Analyses were carried out using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

The study was approved by the CDC and Marshfield Clinic Institutional Review Boards. All adult participants and parents/legal guardians provided written informed consent. All participants were evaluated by a clinician who had no connection to the study and had chosen not to recommend antiviral therapy.

Results

Twenty-one patients with oseltamivir-resistant A (H1N1) infection were randomized during January and February, 2009; two were excluded from the analysis because of lack of compliance with the study drug and clinical symptom reporting. All virus cultures were negative for five participants with a positive RT-PCR result; a 10-year-old girl

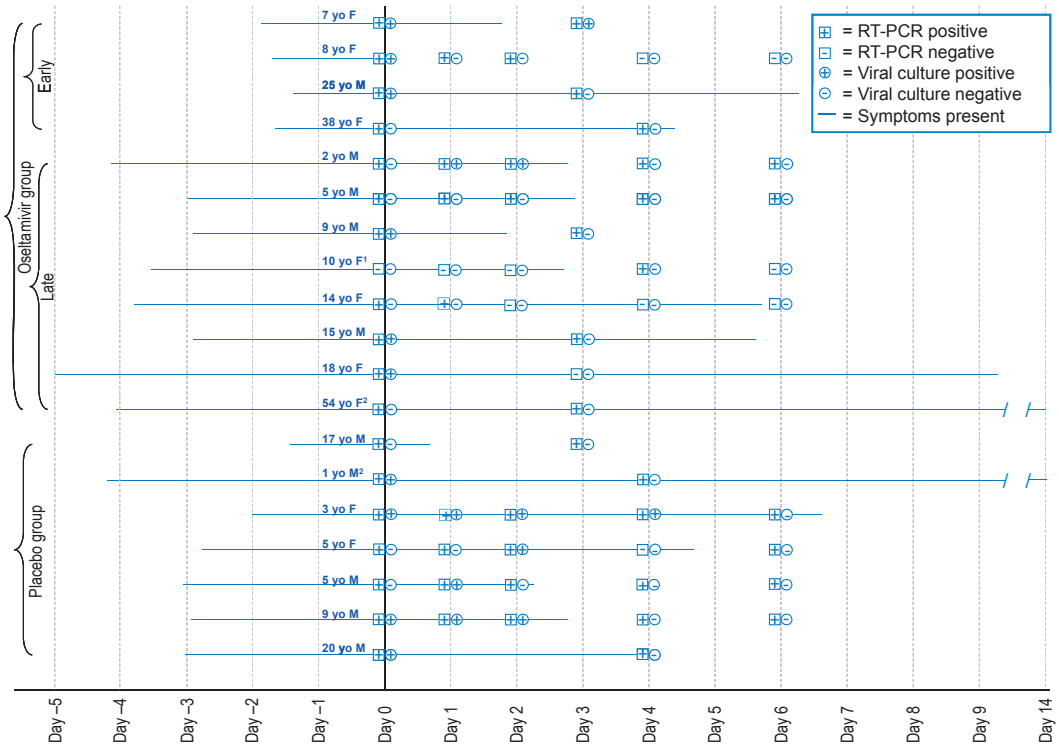

Figure 1. Illness duration and virologic test results for 19 patients with oseltamivir-resistant A (H1N1) infection. [1]Influenza PCR was positive at enrollment, 1 day before randomization; culture was negative. [2]Symptoms did not resolve by day 14.

was PCR positive and culture negative on the day of enrollment, 1 day before randomization (Figure 1; enrollment swabs are not shown). The remaining 14 participants had viruses resistant to oseltamivir by NI with a mean IC_{50} value of 163·9 (standard deviation 70·9) nm. Eighteen viruses or clinical specimens from participants had H275Y by pyrosequencing (100% mutant virus); one clinical specimen was not available for testing.

Most study participants were children or adolescents (Table 1). Among 19 patients, 12 (63%) were randomized to receive oseltamivir and seven received placebo (Figure 1). Four (33%) of the 12 treated patients initiated therapy early and eight (67%) initiated therapy late. Six (86%) of those receiving placebo were randomized >48 hours after illness onset. Nine children participated in the viral shedding substudy (Table 2); 10 provided one follow-up swab on day 3 or 4.

There were no statistically significant differences in duration of viral shedding among the viral shedding substudy participants. However, the median intervals from symptom onset and from time of randomization to first negative virus culture were shorter in the combined oseltamivir treatment group (early and late) compared with the placebo group, and the median interval from randomization to first negative culture was shortest in the early treatment group (Table 2).

No differences in clinical outcomes between treatment groups were statistically significant. However, the median

Table 1. Description of 19 patients with A (H1N1) infection at enrollment

Characteristics	Total *n* = 19 *n* (%)	Oseltamivir All *n* = 12 *n* (%)	Oseltamivir ≤48 hours *n* = 4 *n* (%)	Oseltamivir >48 hours *n* = 8 *n* (%)	Placebo *n* = 7 *n* (%)
Men	10 (53)	5 (42)	1 (25)	4 (50)	5 (72)
Age, median years (range)	9 (1–54)	12 (2–54)	16 (7–38)	12 (2–54)	5 (1–20)
Age groups (years)					
1–<5	3 (16)	1 (8)	0	1 (13)	2 (29)
5–18	12 (63)	8 (67)	2 (50)	6 (75)	4 (57)
19–49	3 (16)	2 (17)	2 (50)	0	1 (14)
>50	1 (5)	1 (8)	0	1 (13)	0
Presence of chronic underlying medical condition*	2 (11)	2 (17)	1 (25)	1 (13)	0
Race/Ethnicity					
White	18 (95)	11 (92)	3 (75)	8 (100)	7 (100)
Unknown	1 (5)	1 (8)	1 (25)	0	0
Household member ill	2 (11)	1 (8)	0	1 (13)	1 (15)
Illness duration prior to randomization, median days (range)	2·9 (1·4–4·9)	3·0 (1·4–4·9)	1·7 (1·4–1·9)	3·7 (2·9–4·9)	2·9 (1·5–4·2)
Clinical characteristics					
Temperature >38°C	2 (11)	1 (8)	0	1 (13)	1 (14)
Fever, subjective	15 (79)	9 (75)	2 (50)	7 (88)	6 (86)
Cough	19 (100)	12 (100)	4 (100)	8 (100)	7 (100)
Sore throat	15 (79)	11 (92)	4 (100)	7 (88)	4 (57)
Nasal congestion	16 (84)	9 (12)	1 (25)	8 (100)	7 (100)
Muscle aches	9 (47)	7 (58)	2 (50)	5 (63)	2 (29)
Wheezing	4 (21)	2 (17)	1 (25)	1 (13)	2 (29)
Fatigue	18 (95)	11 (92)	3 (75)	8 (100)	7 (100)
Headache	13 (68)	9 (75)	3 (75)	6 (75)	4 (57)
Met the Influenza-like Illness (ILI) case definition: fever plus cough or sore throat	15 (79)	9 (75)	2 (50)	7 (88)	6 (86)
Symptom severity score at presentation, median (range)	13 (4–18)	14 (4–18)	12·5 (4–18)	14 (5–16)	12 (8–17)

*The patient was classified as having a chronic underlying medical condition that placed them at high risk for complications of influenza infection if they had ≥2 visits to the Marshfield Clinic during the preceding 12 months that involved an ICD-9-CM diagnosis code in the following chronic disease categories: cardiac disease, pulmonary disease, renal disease, liver disease, diabetes mellitus, immunosuppressive disorders, malignancies, neurologic/musculoskeletal disease, metabolic disease, cerebrovascular disease, and circulatory system disease.

Table 2. Comparison of symptom duration and severity between treatment groups*

Measure	Oseltamivir All *n* = 12	Oseltamivir ≤48 hours *n* = 4	Oseltamivir >48 hours *n* = 8	Placebo *n* = 7
Hours from symptom onset to symptom resolution**, median (range)	166 (50–408)	126 (50–196)	195 (123–408)	179 (61–428)
Hours from randomization to symptom resolution**, median (range)	95 (9–310)	83 (9–163)	111 (53–310)	106 (26–326)
Time to ≥50% decrease in severity score***				
Number of reports	4 (1–17)	3 (1–6)	5 (1–17)	5 (1–10)
In hours	53 (9–202)	38 (9–77)	63 (16–202)	58 (15–120)
Time to resolution of cough†	*n* = 11	*n* = 3		
Number of reports	4 (1–21)	4 (1–6)	4·5 (1–21)	5 (1–14)
In hours	52 (16–252)	52 (18–77)	55 (16–252)	67 (15–166)
Viral shedding substudy (*n* = 9)††	*n* = 5	*n* = 1	*n* = 4	*n* = 4
Days from symptom onset to first negative culture, median (range)†††	*n* = 3	*n* = 1	*n* = 2	*n* = 4
	3 (3–8)	3 (N/A)	5·5 (3–8)	7 (5–8)
Days from randomization to first negative culture, median (range)‡	*n* = 2	*n* = 1	*n* = 1	*n* = 4
	2·5 (1–4)	1 (N/A)	4 (N/A)	4 (2–6)

N/A, not applicable.

**P* values were calculated for the total treated with oseltamivir, treated early with oseltamivir and treated late with oseltamivir groups compared with placebo; all were >0·10.

**Symptom resolution is defined as occurring at the first of two consecutive reports (a ∼ 24 hour period) when all eight symptoms are scored mild or absent (and no decongestant or antitussive products were used during that interval). For two patients that never had symptom resolution (one treated late and one placebo), their last report was assigned as the report at which symptoms were resolved (censored observations).

***Defined as the first of two consecutive reports where severity score was ≤50% of the baseline severity score for the first time.

†Defined as the first of two consecutive reports where cough was scored mild (1) or absent (0) after the first time the highest cough score was reported. One patient (treated early) whose cough severity was scored as mild or absent at all reports was excluded.

††Only nine patients participated in the viral shedding substudy.

†††After last positive result. Two patients that never had a positive culture were excluded (both treated late).

‡After last positive result. Two patients that never had positive culture (both treated late) and one that only had a positive culture at enrollment (treated late) were excluded.

intervals were shorter in the treatment group compared with the placebo group for each of the following parameters: duration of illness (from symptom onset to resolution), interval from randomization to symptom resolution, interval to a 50% reduction in the symptom severity score, and interval from randomization to resolution of cough. Symptom severity scores at enrollment and changes in severity scores over time were similar across the three treatment groups, although symptom severity reported by the early oseltamivir treatment group decreased more rapidly relative to baseline (Figure 2). No patients were hospitalized during the reporting period; follow-up was not conducted after reporting was complete.

Discussion

In this small group of outpatients with oseltamivir-resistant seasonal influenza A (H1N1) infection, we found no statistically significant differences in virologic and clinical outcomes between oseltamivir or placebo treatment groups. Trends suggestive of a shorter duration of clinical symptoms and viral shedding among the groups treated early with oseltamivir were found, but because of our small sample size, these findings may be explained by chance. In addition, these results are not consistent with a limited number of studies that have assessed the effectiveness of oseltamivir treatment in patients with oseltamivir-resistant influenza A (H1N1) infection.[5–7] Our sample size was small because of the unplanned early study termination in the setting of antiviral resistance. We were therefore unable to make any conclusions regarding the correlation of antiviral resistance, as defined by *in vitro* assays, with clinical outcomes.

The determination of resistance to neuraminidase inhibitors among influenza viruses is based on the NI, which determines the IC_{50}, or the concentration of the drug required to inhibit 50% of the virus NA.[2] Reported IC_{50} values for the same virus strain can vary depending on the type of assay used (fluorometric versus chemiluminescent)[8] and the conditions under which the substrate is used.[9] In addition, NI results may not correlate with phenotypic resistance measured by virus growth in cell culture after exposure to a neuraminidase inhibitor;[10] in one study, susceptibility to zanamivir was found in tissue culture while

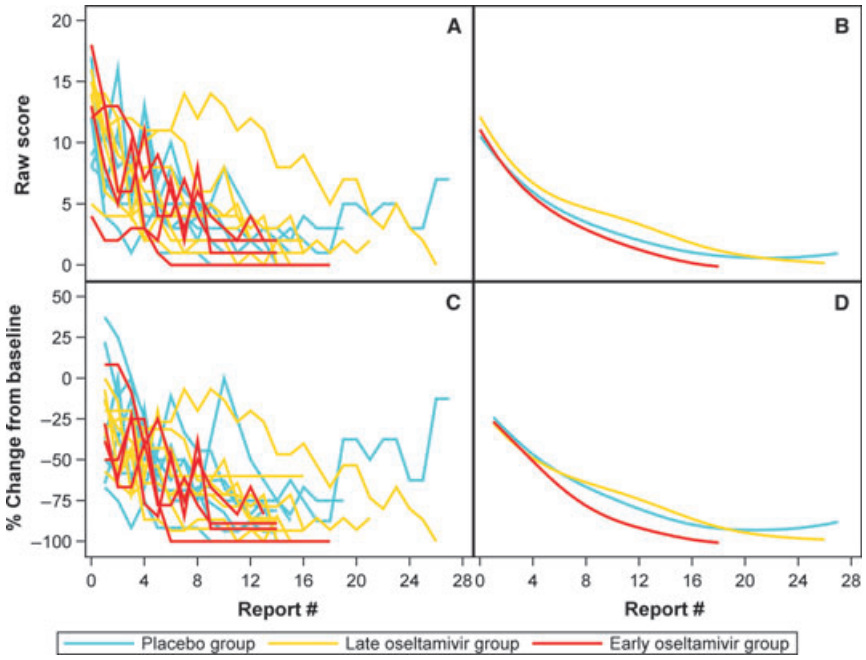

Figure 2. Raw severity scores and percent change from baseline severity scores over time with trend lines by treatment group. Report zero is time of randomization. A. Raw severity scores for each individual. B. Smoothed severity scores by treatment group. C. Raw percent change from baseline severity score for each individual. D. Smoothed percent change from baseline severity score by treatment group.

resistance was detected by NI.[11] It is possible that the effect of a mutation on the ability of a drug to inhibit the NA in the presence of natural substrate may not be completely reflected by *in vitro* assays results. In addition, correlating clinical antiviral effectiveness with *in vitro* resistance might be more difficult in patients with mixed wild-type mutant virus infections. In mixed infections, laboratory testing results are complicated because of selection of wild type or mutant during virus isolation, and clinical responses might vary as the proportion of mutant virus evolves.

The patients in our study had very mild illness, few had fever at presentation, and measuring significant differences in clinical and virologic outcomes would require a large sample size. In our study, the majority of patients receiving placebo were randomized >48 hours after the onset of symptoms, which may bias this group toward a faster resolution of illness; however, the treated groups had shorter durations of clinical symptoms and viral shedding. The IC_{50} values of the viruses infecting subjects in our study varied considerably, and perhaps some were close enough to plasma levels of the active drug, which also can vary,[12,13] to provide modest clinical effect. Also, in one study, oseltamivir was found in middle-ear and sinus fluids with concentrations above 100 mg/l,[14] exceeding the IC_{50} for most resistant viruses.[12]

In lieu of human studies, animal models (e.g., ferrets) may be helpful to evaluate the clinical effects of neuraminidase inhibitors on A (H1N1) viruses containing H275Y. In one study, oseltamivir treatment did not decrease viral titers in ferrets infected with influenza A (H5N1) viruses containing the H275Y mutation[15] and in another, oseltamivir did not affect viral replication in mice infected with 2009 pandemic influenza A (H1N1) viruses with the H275Y mutation.[16] No study reports measured the effect of oseltamivir on illness severity and viral shedding in animals infected with seasonal A (H1N1) viruses with H275Y.

Defining clinical and virologic effects of anti-influenza drugs against resistant viruses, even modest effects, might aid future diagnostic and treatment strategies in the event of wide circulation of a virus with resistance to more than one class of antiviral drugs and support targeted use of molecular assays and investigational drugs for hospitalized and high-risk patients.

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Addendum

N. J. Dharan, E. A. Belongia, A. M. Fry contributed to the concept and design, analysis and interpretation of data, critical writing and revising of the intellectual content, and final approval of the version to be published. B. A. Kieke, M. Vandermause, L. V. Gubareva, A. I. Klimov contributed to the analysis and interpretation of data, critical revising of the intellectual content, and final approval of the version to be published. L. Coleman and J. Meece contributed to

the interpretation of data, critical revising of the intellectual content, and final approval of the version to be published.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

Conflicts of interest

None of the authors have any commercial or other association that might pose a conflict of interest.

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