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Clinical effectiveness of oseltamivir for influenza A(H1N1) virus with H274Y neuraminidase mutation

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Summary *Objective:* To evaluate the clinical effectiveness of oseltamivir therapy started within 48 h of the onset for influenza A(H1N1) virus with H274Y neuraminidase (NA) mutation.
Methods: Virus was isolated before and four to six days after starting oseltamivir treatment from 73 outpatients with influenza A(H1N1) virus in the 2007–2008 and 2008–2009 seasons. NA inhibition assays (IC_{50}) and sequence analyses were done using influenza viruses isolated from these patients. Body temperature was evaluated before and on the second, third, and fourth days after starting treatment.
Results: H274Y mutation was not shown in the 2007–2008 season (44 patients) and shown in all 29 patients in the 2008–2009 season by NA sequence analyses. The mean IC_{50} before oseltamivir treatment was significantly higher in 2008–2009 (319.3 ± 185.4 nM) than in 2007–2008 (1.5 ± 0.8 nM; $p < .001$). Patients ≤ 15 years with oseltamivir-resistant virus infection had a higher ratio of patients persisted virus after oseltamivir treatment than patients > 15 years (50% and 11.8%, respectively, $p = 0.038$), and a significant higher body temperature during oseltamivir treatment, compared to patients ≤ 15 years treated for oseltamivir-sensitive virus infection.
Conclusion: The clinical effectiveness of oseltamivir for the A(H1N1) virus was reduced in the 2008–2009 season compared with the previous season, especially in children, probably due to the H274Y mutation. Oseltamivir seems to be not recommended for children and patients with high-risk underlying diseases infected with H274Y mutated A(H1N1) virus.

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Introduction

The prodrug oseltamivir phosphate (oseltamivir), an oral Neuraminidase (NA) inhibitor, is reported to be effective against influenza infection and is widely prescribed in Japan for the treatment of influenza.[1–5] The duration of fever and viral persistence has previously been studied to monitor clinical effectiveness of oseltamivir for the treatment of influenza.[6–9] Recently, the World Health Organization (WHO) announced that there has been a marked increase in Japan of the oseltamivir-resistant A(H1N1) virus with the N1 NA mutation H274Y (N2 numbering; H275Y in N1 numbering) , from 3% in the 2007–2008 season to 97% in the 2008–2009 season.[10] Japan's Infectious Disease Surveillance Center (IDSC) further announced on February 14, 2009 that 99.5% of A(H1N1) virus isolates in Japan were resistant to oseltamivir.

A 200- to 400-fold reduction in oseltamivir inhibition of NA activity has been observed in vitro with the H274Y mutation.[11–15] However, in Japan, oseltamivir has been commonly used in past seasons against influenza A infection, and was again widely used in the 2008–2009 season, because clinicians were not able to differentiate between influenza A(H1N1) and A(H3N2) viral infections using commercial antigen detection kits. Therefore we were able to evaluate the clinical effectiveness of oseltamivir against the oseltamivir-resistant A(H1N1) strain.

Since April 2009, a novel swine-origin influenza A(H1N1) virus (S-OIA) was identified from the patients in Mexico, USA, Canada or elsewhere.[16–20] On June 11, 2009, the World Health Organization (WHO) raised the worldwide pandemic alert level to Phase 6 in response to the ongoing global spread of the novel influenza A(H1N1) virus. Genetic and phenotypic analysis has indicated that S-OIA was susceptible to oseltamivir and zanamivir but resistant to the adamantanes including rimantadine and amantadine.[16,19,20] However, there are possibilities of S-OIA to become resistant to oseltamivir by mutation of viruses in the near future.

In this study, we investigated presence of H274Y NA mutation, IC_{50} values and body temperature before and after therapy and viral persistence after oseltamivir therapy to evaluate the effectiveness of oseltamivir for influenza A(H1N1) virus with H274Y NA mutation in the 2007–2008 and 2008–2009 influenza seasons in Japan.

Methods

Patients

Patients with influenza-like illnesses with findings such as a body temperature $\geq 37.5\,^{\circ}C$, upper respiratory tract symptoms, and systemic symptoms were tested with antigen detection kits to confirm the presence of influenza A or B in 2007–2008 and 2008–2009 season.[6–9] Family doctors, pediatricians, and physicians at eight clinics participated in the study. 107 patients (2007–2008:60 patients, 2008–2009:47patients) with influenza A diagnosed by commercial antigen detection kits and oseltamivir treatment within 48 h of symptom onset were consecutively enrolled in this study after providing informed consent. And 81 of 107 patients (2007–2008:50 patients, 2008–2009:31 patients) were confirmed influenza A(H1N1) infection by hemagglutinin inhibition (HAI) test. Of 81 patients, 73 patients (2007–2008:44 patients, 2008–2009:29 patients) were analyzed. Eight patients who did not visit the clinic after oseltamivir therapy were excluded from this study. None of the patients had been complicated with other diseases and infected with influenza in the previous season by medical interview or record.

Oseltamivir (adults and children weighing ≥ 37.5 kg: 75 mg; children weighing < 37.5 kg: 2 mg/kg) was administered orally, twice a day, for 5 days. Patients took the initial dose of oseltamivir at a clinic or at home immediately after the diagnosis of influenza by the commercial antigen detection kits. Antipyretics were not administered except acetaminophen as temporally rescue in a few cases.

Age, sex, vaccination status, antigen detection kit test result, and date and time of fever onset were recorded at the first clinic visit. Patients or family members were asked to measure the patient's body temperature at 8:00 AM and 8:00 PM each day. Body temperatures before treatment and at either 8:00 AM or 8:00 PM, whichever was highest, on each of the second, third and fourth days after starting oseltamivir treatment were analyzed.

Antigen detection test kits and virus isolation

Commercial antigen detection kits based on immunochromatography (Capilia FluA+B [Alfresa Pharma], QuickVue Rapid-SP influ [DS Pharma Biomedical] and Imuno Ace Flu [Touns]) were mainly used.

Viruses were isolated before oseltamivir treatment and four to six days after the start of treatment.[8] We calculated the reisolation rates as the ratio of number of patients detected virus at four to six days after starting oseltamivir treatment to number of patients detected virus before treatment. Both of throat and nasal swabs were collected form the patient at the first and the second visit at four to six days after the start of treatment. The swabs were placed in the viral transport medium (Microtest, Multi-Microbe Media, USA). The specimens were shipped to a central laboratory (Mitsubishi Chemical Medience Co., Tokyo, Japan) and viral isolation was done by the standard method using Madin-Darby canine kidney (MDCK) cells (DS Pharma Biomedical Co., Ltd., Osaka, Japan). The influenza A(H1N1) subtype of the isolated viruses were determined by HAI test with serum HAI antibodies (Denka Seiken Co., Ltd, Tokyo, Japan).[8]

NA inhibition assay

The sensitivity of the viral NA to oseltamivir carboxylate (F. Hoffmann-La Roche Ltd, Basel, Swiss Confederation) was assessed with an NA enzyme inhibition assay based on the method reported by Gubareva et al.[21,22] The isolated viruses were shipped to overseas laboratory (ViroClinics BV, Rotterdam, Netherlands), and NA inhibition assay was executed to determine the 50% inhibitory concentration (IC_{50}) for oseltamivir carboxylate. In brief, the isolated viruses and the control viruses were treated with NP-40 and incubated in the presence of serial dilutions (from