2. Autoimmune Myocarditis (Myocarditis Derived From Collagen Diseases)

1 Background

Initial manifestations of autoimmune myocarditis include dysfunction of the kidneys, skin, choroid plexus, and inflammation not involving infection such as deposition of immune complexes and activation of complement.

2 Diagnosis

Initially, autoimmune myocarditis rarely occurs with myocarditis alone. Pericarditis is associated with the severity of disease activity. A presence of antinuclear antibodies in pericardial effusion, autoantibodies, reduced complement activity, and elevated immune complex levels are supportive of the diagnosis. Echocardiography, myocardial scintigraphy and CMR imaging are used for diagnosis, but an ordinary endomyocardial biopsy is not diagnostic. Scleroderma, systemic lupus erythematosus, polymyositis, dermatomyositis, rheumatoid arthritis, polyarteritis nodosa, and allergic granulomatous angiitis (Churg-Strauss syndrome) are associated with cardiac manifestations.[41]

3 Treatment

Autoimmune myocarditis is treated with corticosteroids or concurrent use of immunosuppressant if the patient has decreased cardiac function, severe pericardial effusion, or concurrent dysfunction of other organs.

3. Drug-Induced Myocarditis

1 Background

This type of myocarditis is induced by drug treatment. Myocarditis may occur in any patient treated with drugs, and must be carefully monitored. Hypersensitivity myocarditis occurs a few days to a few months after exposure to a drug regardless of the dosage used.[42] The severity of toxic myocarditis depends on the dosage and method of administration and drug metabolism in patients, and the onset of this type of myocarditis is slow.

2 Diagnosis

An endomyocardial biopsy is of crucial importance to the diagnosis of drug-induced myocarditis. However, diagnosis also requires information such as history of drug treatment and clinical conditions after discontinuation of the suspected drug, since differentiation of drug-induced myocarditis from acute myocarditis of other etiologies is difficult. Blood cardiac troponin is useful for diagnosis. Although hypersensitivity myocarditis presents with an elevated eosinophil count, the usefulness of the eosinophil count in diagnosis is unclear.

3 Treatment

Discontinuation of the suspected drug is the most effective method of treatment. Treatment with corticosteroids is also expected to be useful for hypersensitivity myocarditis and drug-induced hypersensitivity syndrome.[43] After the patient recovery, re-administration of the suspected drug must be prevented.

IV Conclusion

The diagnosis of myocarditis is difficult. The first step in diagnosis is to suspect myocarditis. The primary principles of treatment are to make the clinical diagnosis and manage cardiopulmonary emergency promptly. Every effort must be made to confirm the diagnosis of myocarditis by histology, since some cases of specific myocarditis may respond to corticosteroid treatment.

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Appendix

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MRI Is Useful for Diagnosis of H1N1 Fulminant Myocarditis

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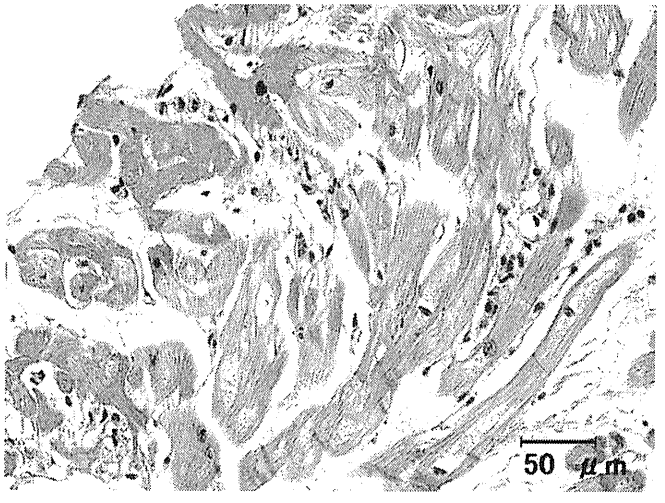

Figure 1. Cardiac biopsy from the left ventricular posterior wall shows only mild inflammation and degeneration of myocytes, though the patient was in cardiac shock. Bar=50μm.

A 28-year-old Japanese man complained of fever (body temperature 39.2°C) and visited a clinic, where he was given oral oseltamivir for 5 days.[1] One week later, he revisited the clinic because of chest pain and respiratory discomfort (36.2°C). Because he was in shock (systolic blood pressure: 80 mmHg, heart rate: 110 beats/min), he was transferred to the Emergency Medical Center. Blood examination on admission showed elevated levels of creatine phosphokinase (2,540 U/L), and troponin I (13.5 ng/ml), and echocardiography revealed diffuse hypokinesis and an ejection fraction of 22%. He was positive for H1N1 virus on polymerase chain reaction performed on admission. Because of his state of shock, an intra-aortic balloon pump (IABP) was inserted.[2] Emergency coronary angiography did not show stenosis of the right or left coronary artery, but left ventriculography revealed diffuse hypokinesis. Because a differential diagnosis was fulminant myocarditis, 3 biopsy samples were taken from the left ventricular posterior wall, but each of the serial cardiac biopsy sections showed only mild inflammation[3] (**Figure 1**). His cardiac function improved with supportive therapy and 3 days after admission, the IABP was removed. On magnetic resonance imaging (MRI; Signa CV/i 1.5 Tesla ver. 9.1 (GE Medical Systems, USA) TR 2,000 ms, TE 80 ms) performed 4 days after admission, the T2-weighted image (taken before enhancement) showed remarkable myocardial inflammation in the region centered around the left ventricular posterior wall and apex[4] (**Figure 2**). To check myocardial blood flow, we performed MRI with contrast media, but there were no abnormalities.[5,6] Therefore, the high intensity on MRI was thought to indicate inflammation (**Movie 1**).

In conclusion, MRI might be more useful than invasive cardiac biopsy for diagnosing myocarditis caused by H1N1 influenza and to estimate the activity and severity of the inflammation.

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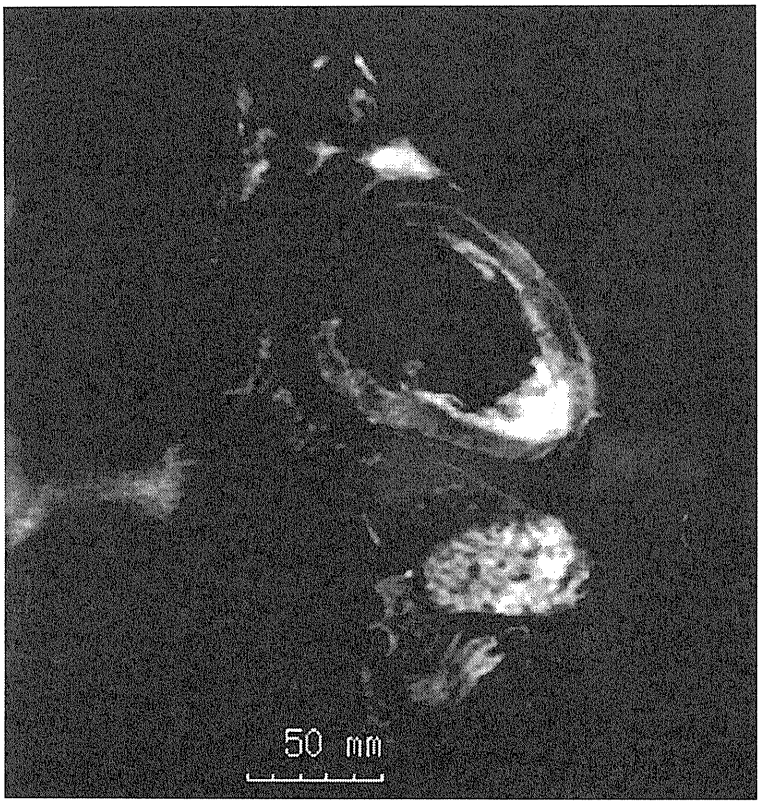

Figure 2. T2-weighted magnetic resonance imaging (MRI) shows remarkable myocardial inflammation in the region of the left ventricular posterior wall and apex, whereas the serial biopsy sections showed only mild inflammation.

Disclosure

There is no potential conflict of interest.

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Supplementary files

Movie 1. CINE-MRI: Steady-state gradient echo (steady-state free precession).

Please find supplementary file(s);
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ORIGINAL ARTICLE

In vitro neuraminidase inhibitory activities of four neuraminidase inhibitors against influenza viruses isolated in the 2010–2011 season in Japan

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Abstract The half maximal inhibitory concentration (IC_{50}) of four neuraminidase inhibitors (NAIs), oseltamivir, zanamivir, laninamivir, and peramivir; was measured using influenza viruses isolated in the 2010–2011 influenza season in Japan. Clinical samples for viral isolation were obtained from nasal aspiration, nasopharyngeal swab, or self-blown nasal discharge and cultured with Madin–Darby canine kidney cells. The type and subtype of H3N2 or B were determined by reverse transcriptase polymerase chain reaction (RT-PCR). For the A(H1N1)pdm09 virus, the subtype was determined by real-time RT-PCR. IC_{50}s to oseltamivir carboxylate, zanamivir, laninamivir, and peramivir were determined by a fluorescence-based neuraminidase inhibition assay. Influenza viruses were isolated from 269 patients. A(H1N1)pdm09, H3N2, and B were isolated from 185, 54, and 30 patients, respectively. The geometric means of IC_{50} for oseltamivir were 0.86 and 0.73 nM to A (H1N1) pdm09, except for the two outlier viruses described below and H3N2, respectively, and 33.12 nM for B. The geometric means of IC_{50} for the other three NAIs were lowest to A(H1N1)pdm09 and highest to B. Two A(H1N1)pdm09 isolates showed very high IC_{50} values for oseltamivir (840 and 600 nM) and peramivir (19 and 24 nM). No isolate showed significantly high IC_{50} values for zanamivir or laninamivir. Continuous surveillance against the emergence or spread of influenza virus with high IC_{50} values for anti-influenza drugs is important.

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Keywords Influenza · Half maximal inhibitory concentration (IC_{50}) · Oseltamivir · Zanamivir · Laninamivir · Peramivir

Introduction

Treating influenza with neuraminidase inhibitors (NAIs) has become the most popular treatment among primary care doctors in Japan. A swine-origin H1N1 strain, A(H1N1) pdm09, was the cause of a pandemic in 2009 [1]. Fortunately, the number of reported influenza-associated deaths was only about 200 in Japan, far fewer than in other countries [1]. The early start of treatment with NAIs, within 48 h of the onset of the influenza symptoms, may have contributed to mitigating symptoms and preventing severe disease.Two NAIs, oseltamivir (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) and zanamivir (GlaxoSmithKline K.K. Tokyo, Japan), are commonly used in Japanese clinics. The clinical effectiveness of anti-influenza drugs has been confirmed in clinical settings [2–4]. Recently, two new NAIs, laninamivir (Daiichi Sankyo Co., Ltd., Tokyo, Japan) and peramivir (Shionogi & Co., Ltd., Osaka, Japan), were added to the options for influenza treatment in Japan. However, as these various NAIs have been available in the market, drug resistance has become of important clinical concern. An A/H1N1 oseltamivir-resistant strain with a mutation at position 275 of NA was reported in Europe in 2007, and it quickly spread throughout the world [5]. Almost all seasonal A/H1N1 viruses have acquired resistance to oseltamivir worldwide [6]. It has been reported that the H275Y mutant reduces sensitivity to oseltamivir by several hundred-fold in vitro [7]. Reduced clinical effectiveness of oseltamivir to H275Y mutated H1N1 viruses compared to the wild-type H1N1 seasonal influenza virus has been confirmed in the clinical

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setting [8, 9]. In addition, the emergence of H275Y mutated A(H1N1)pdm09 with resistance to oseltamivir has been reported [10]. To study the extent of drug resistance, we surveyed the half maximal inhibitory concentration (IC_{50}) of four NAIs, oseltamivir, zanamivir, laninamivir, and peramivir, from influenza viruses isolated in the 2010–2011 influenza season in Japan. The results, including two A(H1N1)pdm09 isolates with significantly high IC_{50} values for oseltamivir and peramivir, but not for zanamivir and laninamivir, are reported.

Materials and methods

Patients

A total of 22 clinics and hospitals from 13 prefectures in Japan participated in this study. Patients were enrolled from 1 November 2010 to 30 April 2011. Samples for viral isolation were collected from patients who showed a positive result by rapid influenza antigen detection kits, based on immunochromatography, with informed consent.

Influenza virus isolation

Clinical samples for viral isolation were obtained from nasal aspiration, nasopharyngeal swab, or self-blown nasal discharge. Samples were suspended with a solution for virus preservation (M4-RT medium, Remel, KS, USA) and sent to a central laboratory (Mitsubishi Chemical Medience Corporation) where they were kept at −80°C. The collected samples were cultured with Madin–Darby canine kidney (MDCK) cells at 33°C.

Viral types and subtypes

The type and subtype of H3N2 or B was determined by amplified DNA size of reverse transcriptase polymerase chain reaction (RT-PCR) using type- and subtype-specific primers as described [11]. In brief, viral RNA was extracted from the clinical sample, then complementary DNA (cDNA) was synthesized using reverse transcriptase. PCR was done with cDNA using primer sets specific for viral type and subtype. For the A(H1N1)pdm09 virus, the subtype was determined by real-time RT-PCR with a specific primer set and a fluorescent-labeled probe (http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/index.html).

Measurement of IC_{50} of NA inhibitors

IC_{50}s to oseltamivir carboxylate, zanamivir, laninamivir, and peramivir were determined by a fluorescence-based neuraminidase inhibition assay with culture supernatants, as described elsewhere [12]. Laninamivir and zanamivir were provided by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Oseltamivir carboxylate was prepared from oseltamivir phosphate extracted from the commercial preparation Tamiflu® (Chugai Pharmaceutical Co., Ltd., Tokyo, Japan). Peramivir was obtained from the commercially available product (Rapiacta®, Shionogi & Co., Ltd., Osaka, Japan).

Statistical analysis

Difference in age distribution among A(H1N1)pdm09, H3N2, and B patient groups was tested by analysis of variance (ANOVA). Quantitative data were tabulated to provide descriptive summary statistics. Geometric means and 95% confidence intervals (CI) were calculated for IC_{50} values. Box and whisker plots were drawn with log-transformed IC_{50} values by influenza type and subtype. For A(H1N1)pdm09, scatter plots of log-transformed IC_{50} values were made to compare the IC_{50} values of each NAI. P value <0.05 was considered statistically significant. All analyses were performed by SAS® System Release 8.2 (SAS Institute, Cary, NC, USA).

Results

A total of 289 influenza-kit-positive patients were enrolled. Among them, 269 influenza viruses were isolated. Influenza virus A(H1N1)pdm09, H3N2, and B were isolated from 185, 54, and 30 patients, respectively. Age distribution of the patients by virus type and subtype is listed in Table 1. The mean age of the 269 patients who had a virus isolated was 28.1 ± 17.1 years. There was no significant difference in mean ages between males and females. The mean age of A(H1N1)pdm09-positive patients was 30.0 ± 16.2 years, higher than that of H3N2 and B (23.1 ± 18.4 and 21.2 ± 16.5 years, respectively). The difference of age distribution between patients with A(H1N1)pdm09 and H3N2 or B infection was statistically significant ($P = 0.0009$).

The geometric mean of IC_{50} for the four NAIs is listed in Table 2. The geometric mean of IC_{50} for oseltamivir was 0.86 and 0.73 nM to A(H1N1)pdm09, except for the two outlier viruses described below and H3N2, respectively; and 33.12 nM for B. The geometric mean of IC_{50} for the other three NAIs was lowest to A(H1N1)pdm09 and highest to B. The ratio of IC_{50} for B to that of H3N2 for oseltamivir was 45.4 and for zanamivir, laninamivir, and peramivir were 6.8, 6.6, and 6.0, respectively.

The distribution of IC_{50} of the four NAIs is depicted in Fig. 1. The $\log_{10}$ (IC_{50})s of each NAI were distributed in a

Table 1 Distribution of patients by age, sex and virus type

Age group	No. of patients	Males	Females	A(H1N1) pdm09	H3N2	B
0–9	33	14	19	14	13	6
10–19	65	41	24	36	17	12
20–29	54	30	24	43	5	6
30–39	43	24	19	34	6	3
40–49	38	22	16	30	7	1
50–59	25	12	13	22	3	0
60–69	8	2	6	4	3	1
70–79	3	2	1	2	0	1
80+	0	0	0	0	0	0
Total	269	147	122	185	54	30
Mean age ± SD (years)	28.1 ± 17.1	27.5 ± 16.4	28.8 ± 18.0	30.0 ± 16.2	23.1 ± 18.4	21.2 ± 16.5

Data are shown as the number of mean ± standard deviation

Table 2 Half maximal inhibitory concentration (IC_{50}) values of four neuraminidase inhibitors (NAIs) for viral isolates from the 2010–2011 influenza season in Japan

Drug	Geometric mean IC_{50} (nM)		
	A(H1N1)pdm09 (n = 185) Geometric mean (95% CI)	H3N2 (n = 54) Geometric mean (95% CI)	Influenza B (n = 30) Geometric mean (95% CI)
Oseltamivir	0.86 (0.76–0.98)	0.73 (0.65–0.82)	33.12 (28.78–38.09)
Zanamivir	0.73 (0.69–0.78)	1.64 (1.51–1.79)	11.21 (9.98–12.61)
Laninamivir	1.37 (1.27–1.47)	3.22 (2.91–3.56)	21.25 (19.12–23.64)
Peramivir	0.38 (0.34–0.42)	0.66 (0.61–0.71)	3.96 (3.44–4.55)

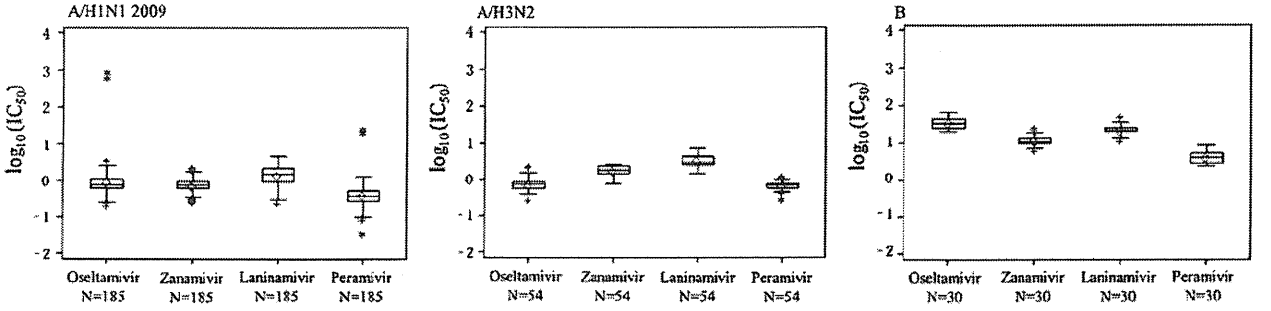

Fig. 1 Half maximal inhibitory concentration (IC_{50}) quartiles of each neuraminidase inhibitor (NAI) for different influenza types. *Diamond* arithmetic mean, *plus symbol* values between 1.5 × IQR and 3 × IQR from UQ/LQ; *asterisk* values above/below 3 × IQR from UQ/LQ, respectively. *IQR* interquartile range, *UQ* 75 percentile, *LQ* 25 percentile

narrow range, except for two viral isolates of A(H1N1)pdm09. The two A(H1N1)pdm09 isolates showed very high IC_{50} values for oseltamivir (840 and 600 nM) and peramivir (19 and 24 nM).

Scatter plots of the log-transformed IC_{50} values of each NAI are shown in Fig. 2. Two isolates showed very high IC_{50} values for oseltamivir but not for zanamivir (Fig. 2a) or laninamivir (Fig. 2b). Two isolates showed high IC_{50} values for both oseltamivir and peramivir (Fig. 2c). No isolate showed a very high IC_{50} value for zanamivir or laninamivir (Fig. 2d). Two isolates showed very high IC_{50} values for peramivir but not for zanamivir (Fig. 2e) or laninamivir (Fig. 2f).

Discussion

In the 2010–2011 season, three influenza strains, A(H1N1) pdm09, H3N2, and B were epidemic in Japan. In this study, A(H1N1)pdm09 was responsible for 68.8% of the isolated viruses. In the 2009–2010 season, almost all clinical isolates were reported to be A(H1N1)pdm09, and patients were mainly 19 years of age and younger. In this study, almost 30% of the patients with A(H1N1)pdm09 were in this age group. The reason for change in the rate of A(H1N1)pdm09 patients in this age group is unknown. For the four NAIs, there was a tendency for the IC_{50} of influenza B virus to be higher than that of A(H1N1)pdm09 and H3N2. The ratio of

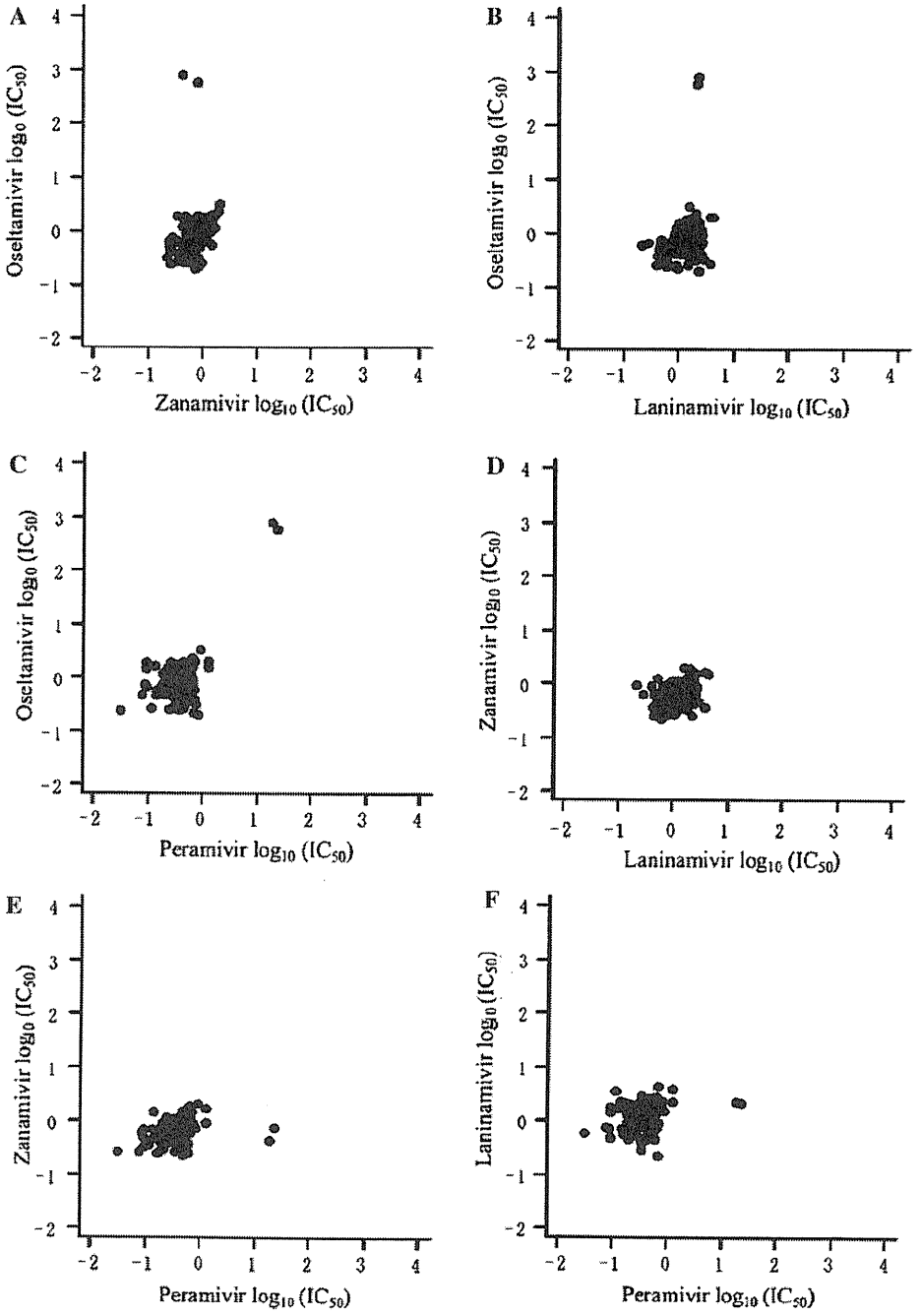

Fig. 2 Scatter plots of Half maximal inhibitory concentration (IC_{50}) values of the four neuraminidase inhibitors (NAIs) for A(H1N1)pdm09

IC_{50} for B to that of H3N2 was especially high in oseltamivir compared with the other three NAIs. It has been reported that the clinical effectiveness of oseltamivir is inferior to influenza B in comparison with influenza A [2]. The clinical efficacy of each drug has not been evaluated in this study. It is plausible that the IC_{50} value or ratio of IC_{50} to viral type and subtype may be useful for predicting the clinical effectiveness of each NAI to a certain viral type or subtype. Further study is necessary to ascertain a relationship between clinical efficacy and IC_{50} value.

The prevalence of oseltamivir-resistant virus was reported to be 1.0% in the 2009–2010 influenza season (http://idsc.nih.go.jp/iasr/graph/tamiful09-10.gif). In this study, two A(H1N1)pdm09 isolates displayed high IC_{50} values for oseltamivir, and the prevalence of oseltamivir resistant virus was calculated at 0.74% of all isolates and 1.1% of A(H1N1)pdm09 isolates. No significant increase in oseltamivir-resistant A(H1N1)pdm09 was observed. However, the existence of oseltamivir-resistant viruses is important; thus, continuous surveillance is necessary. Two

A(H1N1)pdm09 isolates displayed high IC_{50} values for oseltamivir and peramivir, but not for zanamivir and laninamivir. The emergence of A(H1N1)pdm09 viruses with high IC_{50} values has been reported for pediatric patients treated with oseltamivir (http://idsc.nih.go.jp/iasr/rapid/pr3641.html, in Japanese). The molecular basis for H275Y resistance to N1 was described in a structural study of the mutant enzyme [13]. Conformational change induced by the H275Y mutation may affect the binding of N1 neuraminidase, not only to oseltamivir but to peramivir [14]. Further study is necessary to investigate clinical impact correlating increased IC_{50} values.

In conclusion, A(H1N1)pdm09, H3N2, and B were prevalent in the 2010–2011 season in Japan, with A(H1N1)pdm09 being dominant. Of the A(H1N1)pdm09 isolates, two of 269 displayed high IC_{50} values for oseltamivir and peramivir. No isolates displayed significantly high IC_{50} values for zanamivir and laninamivir.

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ORIGINAL ARTICLE

Persistence of pandemic influenza H1N1 virus in young patients after oseltamivir therapy in the 2009–2010 season: a comparison with seasonal H1N1 with or without H275Y mutation

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Abstract Comparison of the viral persistence of pandemic H1N1 (H1N1pdm) and seasonal H1N1 with or without H275Y mutation after oseltamivir therapy has not been adequately done. Virus was isolated before and on days 4–6 from the start of oseltamivir treatment for 158 cases of seasonal (2007–2008 and 2008–2009 seasons) or pandemic (2009–2010 season) H1N1 influenza. Sequence analysis was done for each season and NA inhibition assay (IC_{50}) was done in the 2009–2010 season. H275Y mutation before therapy was 0% in the 2007–2008 and 2009–2010 seasons, but 100% in the 2008–2009 season. Fever and other symptoms were noticeably prolonged after oseltamivir therapy for children with H275Y mutated seasonal H1N1 (2008–2009 season), but not in patients with seasonal H1N1 without mutation (2007–2008) or H1N1pdm (2009–2010). The viral persistence rate was significantly higher for patients 15 years or younger than for those 16 years and older with H275Y mutated seasonal H1N1 (46.2% and 10.5%, respectively) or with H1N1pdm (43.3% and 11.5%, respectively). The H275Y mutation emerged after oseltamivir treatment in 2.4% (2/82) of all patients with H1N1pdm. In two children, the H275Y mutation emerged after therapy and the IC_{50} increased more than 200 fold; however, the prolongation of fever was not so prominent. In conclusion, oseltamivir was effective for fever and other clinical symptoms; however, the virus persisted longer than expected after treatment in H1N1pdm influenza-infected children in the 2009–2010 season, similar to seasonal H1N1 with H275Y mutation in the 2008–2009 season.

Keywords Influenza A(H1N1) · Oseltamivir · Viral shedding · H275Y mutation · IC_{50}

Introduction

The prodrug oseltamivir phosphate (oseltamivir), an oral neuraminidase (NA) inhibitor, had been effective against seasonal influenza A infection until the 2007–2008 season [1–7], but became less effective in the 2008–2009 season, when seasonal H1N1 influenza with the H275Y mutation [8, 9] was widespread throughout Japan [10, 11]. The reduction was more prominent in children than in adults [10, 11], however, oseltamivir was effective for the pandemic H1N1 (H1N1pdm) influenza that emerged in 2009, as shown by our previous study estimating the duration of fever after the start of oseltamivir therapy [12].

There is great concern about the length of the viral shedding period after oseltamivir therapy for H1N1pdm infection, because longer viral shedding holds the possibly of inducing secondary infection in the home or community. A longer viral shedding period may also be related to the emergence of viral mutation of H1N1pdm viruses [7, 10, 13]. Although the frequency of H275Y mutation of

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H1N1pdm virus was reported to be very low in the 2009–2010 season [13, 14], the rapid emergence of oseltamivir-resistant H1N1pdm virus during oseltamivir therapy has been recently reported in hospitalized patients [15, 16]. Viral persistence and the emergence of H275Y mutation during oseltamivir therapy for H1N1pdm has not been adequately analyzed in outpatients.

In this study, we investigated the persistence of symptoms and viruses and the emergence of H275Y NA mutation after oseltamivir therapy for Japanese H1N1pdm patients in a comparison with seasonal H1N1 with or without H275Y mutation. IC_{50} values were also calculated for H1N1pdm before and after therapy.

Methods

Patients

Patients with influenza-like illnesses with findings such as body temperature ≥37.5°C, upper respiratory tract symptoms, and systemic symptoms were tested with antigen detection kits to confirm the presence of influenza A or B in the 2007–2008, 2008–2009 and 2009–2010 seasons. Family doctors, pediatricians, and physicians at 8 clinics (1 clinic each in Gifu, Kumamoto, Gunma, Kanagawa, and Tokushima Prefectures and 3 clinics in Ishikawa Prefecture) in the 2007–2008 and 2008–2009 seasons and at 11 clinics (1 clinic each in Gifu, Kumamoto, Gunma, Kanagawa, and Tokushima Prefectures and 3 clinics each in Ishikawa and Fukuoka Prefectures) in the 2009–2010 season participated in the study. We enrolled, in this study, consecutively, 204 patients (2007–2008, 59 patients; 2008–2009, 54 patients; 2009–2010, 91 patients) with influenza A diagnosed by commercial antigen detection kits who received oseltamivir treatment within 48 h of symptom onset after obtaining informed consent; 170 of 204 patients (47 in 2007–2008; 34 in 2008–2009; 89 in 2009–2010) had influenza A(H1N1) infection confirmed by hemagglutinin inhibition (HAI) test; 12 patients who did not visit the clinic after oseltamivir therapy were excluded from the study, leaving the data of 158 (44 in 2007–2008; 32 in 2008–2009; 82 in 2009–2010) available for analysis. None of the patients had complications from other diseases.

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 to all patients. Oseltamivir has been reported to be related to the neuropsychiatric symptoms of young adults and has been prohibited, in most cases, for use by patients aged from 10 to 19 years in Japan. A warning letter concerning the neuropsychiatric symptoms possibly induced by oseltamivir in young adults appeared on the following website (in Japanese): http://www.mhlw.go.jp/houdou/2007/03/h0320-1.html. Therefore, the decision to administer oseltamivir was left to the discretion of the clinician, who followed the foregoing guidelines and patient preference. Patients took the initial dose of oseltamivir at a clinic or at home immediately after the diagnosis of influenza by a commercial antigen detection kit. Antipyretics were not administered, except for acetaminophen, which was used temporally 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 a.m. and 8:00 p.m. each day. Body temperature before treatment or at either 8:00 a.m. or 8:00 p.m., whichever was highest, on days 2, 3, and 4 after the start of oseltamivir treatment was analyzed. Patients or family members were also asked to record, at 8:00 a.m. and 8:00 p.m. each day, a symptomatic score (score 0, none; score 1, mild; score 2, moderate; score 3, severe) for six clinical symptoms: nasal symptoms (rhinorrhea or nasal obstruction), cough, sore throat, myalgia or joint pain, general fatigue, and headache.

Antigen detection test kits and virus isolation

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

Viruses were isolated before oseltamivir treatment and on days 4–6 after the start of treatment [7]. We calculated the persistence rate as the ratio of the number of patients in whom virus was detected on days 4–6 after the start of oseltamivir treatment to the number of patients for whom the virus was detected before treatment. Nasopharyngeal swabs were collected from the patient at the first and the second visits, on days 4–6 after the start of treatment. The swabs were placed in viral transport medium (Microtest, Multi-Microbe Media, USA). Viral isolation was done by the standard method using Madin–Darby canine kidney (MDCK) cells (DS Pharma Biomedical, Osaka, Japan). The influenza A(H1N1) subtype of the isolated viruses were determined by HAI test with serum HAI antibodies (Denka Seiken, Tokyo, Japan). The virus isolation and HAI test were performed by Mitsubishi Chemical Medience, Tokyo, Japan.

NA inhibition assay

Viral sensitivity to inhibition by oseltamivir carboxylate (OC) (F. Hoffmann-La Roche, Basel, Swiss Confederation) was determined by phenotyping, using a NA-Star

chemiluminescent substrate-based NA enzyme assay. This phenotyping assay has been well established and is widely used as part of ongoing global influenza surveillance programs [17, 18]. A detailed description of the assay principles and performance can be found on the website of the Neuraminidase Inhibitor Susceptibility Network (NISN): http://www.nisn.org/v_ic50_methodology.html or applied biosystems: http://www.appliedbiosystems.jp/website/CONTENTS/NA-Star_protocol.pdf. The phenotyping assay was performed by ViroClinic, Rotterdam, The Netherlands.

NA sequence analysis

MDCK culture aliquots were shipped to RIKEN Omics Science Center (RIKEN Yokohama Institute, Japan) where reverse transcription-polymerase chain reaction (RT-PCR) and sequencing of the NA gene [19] were done. Viral RNA was successfully amplified from the baseline sample, and the rgw NA sequence was consistent with pandemic influenza A(H1N1). Extracted RNA was transcribed into cDNA by multi-segment RT-PCR with 5′-ACGCGTG ATCAGCAAAAGCAGG-3′ and 5′-ACGCGTGATCAG TAGAAAGG-3′ [19]. For sequencing of the pandemic 2009 N1NA gene, corresponding cDNAs were amplified by PCR using 5′-ACGCGTGATCAGCAAAAGCAGG-3′ (forward) and 5′-ATTAGGGTTCGATATGGGCT-3′ (reverse) primers with the first cDNA fragment, 5′-CC TTGGAATGCAGAACCTTC-3′ (forward) and 5′-GATT GTCTCCGAAAATCCCA-3′ (reverse) primers with the second fragment, 5′-AAAGGGAAAGATAGTCAAAT-3′ (forward), and 5′-ACGCGTGATCAGTAGAAACAAGG-3′ (reverse) primers with the third fragment.

Statistical analysis

The Mann–Whitney U test was used for between-group comparisons of median values concerning age, body temperature, total symptom score, IC_{50}, time from onset of symptoms to sampling, and the interval between the first and second virus sampling. Fisher's exact test was also done to compare between group percentages of the persistence rates of virus, male-to-female ratio, and vaccination status. $P < 0.05$ was considered statistically significant.

Results

Patient characteristics and H275Y mutation before therapy

Of 158 patients with influenza A(H1N1) virus infection, 44 presented during the 2007–2008 season (December 1, 2007–February 27, 2008), 32 during the 2008–2009 season (December 1, 2008–April 30, 2009), and 82 during the 2009–2010 season (November 1, 2009–April 30, 2010). No H275Y mutation was detected before therapy by NA sequence analysis in seasonal H1N1 in 2007–2008 or in H1N1pdm in 2009–2010, but in all seasonal H1N1 in 2008–2009. Patient demographic characteristics for seasonal H1N1 without H275Y mutation (2007–2008), seasonal H1N1 with H275Y mutation (2008–2009), and H1N1pdm (2009–2010) are summarized in Table 1. No significant pretreatment differences among the groups were found for median values of age, body temperature, or total symptom score, male-to-female ratio, or vaccination status. The median (25th–75th percentile) time from onset of symptoms to sampling was 13.8 (7.1–22.1) h in the 2007–2008, 19.7 (12.8–29.9) h in the 2008–2009, and 19.8 (14.0–26.9) h in the 2009–2010 seasons (2007–2008 vs. 2008–2009, $P = 0.071$; 2007–2008 vs. 2009–2010, $P = 0.010$; 2008–2009 vs. 2009–2010, $P = 0.962$).

Body temperature before and after the start of therapy

Figure 1 shows the mean value of the highest body temperature on day 1 (before therapy), and days 2, 3, and 4 after starting oseltamivir therapy for seasonal H1N1 with or without H275Y mutation and for H1N1pdm.

For adults 16 years and over, the mean values of fever of all three groups declined to less than 37°C on day 3 or 4 after starting oseltamivir therapy. For children 15 years and under, the mean value of fever declined to less than 37°C on day 3 or 4 in seasonal H1N1 without H275Y mutation, but remained greater than 37°C on day 3 or 4 in seasonal H1N1 with the H275Y mutation. In H1N1pdm, the mean value of fever declined to under 37°C on day 3 or 4 in children, similar to seasonal H1N1 without H275Y mutation.

Persistence of other symptoms after therapy

The persistence rate of symptoms was calculated as the number of patients with each symptom at the second virus sampling on days 4–6 after the start of therapy divided by the number of patients in each patient group.

The persistence rates of the six symptoms for seasonal H1N1 without (2007–2008) or with (2008–2009) H275Y mutation and H1N1pdm (2009–2010) were 7.1% (1/14), 61.5% (8/13), and 30% (9/30), respectively ($P = 0.004$ between 2007–2008 and 2008–2009), for children 15 years and younger. The rates for adults 16 years and older were 36.7% (11/30), 42.1% (8/19), and 32.7% (17/52), respectively, with no significant differences among the three groups.

Table 1 Baseline demographic characteristics of patients with seasonal or pandemic H1N1 influenza

	Seasonal H1N1		H1N1pdm (2009–2010)
	H275Y mutation (–) (2007–2008)	H275Y mutation (+) (2008–2009)	
n	44	32	82
Age (years)[a]	33.5 (7–41.3)	24.5 (5.5–31.8)	25.5 (8.3–39.8)
Male/female	27/17	14/18	37/45
Vaccination[b] (positive/negative/unknown)	10/34/0	11/21/0	26/54/2
BT before therapy (°C)[a]	38.3 (37.7–38.8)	38.0 (37.4–38.8)	38.2 (37.8–38.7)
Total symptom score[a,c]	8 (6–11)	8 (5–10)	7 (4–10)

No significant difference was found in any of the parameters for the 2007–2008, 2008–2009, and 2009–2010 seasons

BT body temperature

[a] Median (25–75 percentile)

[b] Vaccination, vaccination for seasonal influenza

[c] Total symptom score, total of the individual scores for the following six symptoms: nasal symptoms, cough, sore throat, myalgia or joint pain, general fatigue, and headache (score 0, none; score 1, mild; score 2, moderate; score 3, severe)

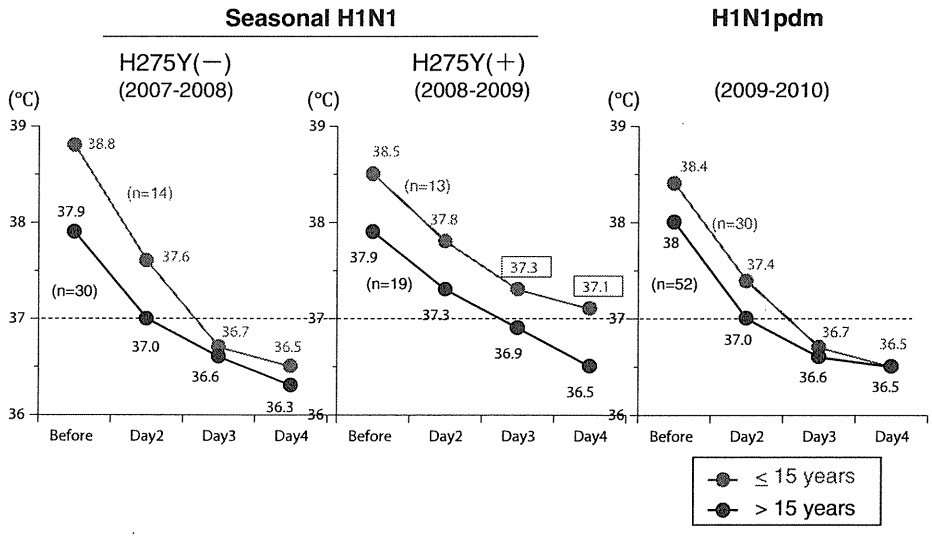

Fig. 1 Mean body temperature before, and on days 2, 3, and 4 after, the start of therapy in the 2007–2008, 2008–2009, and 2009–2010 seasons. Mean body temperature above 37.0°C was seen not only before day 2, but also on day 3 or 4 (*numbers enclosed in boxes*) in children in the 2008–2009 season in which H1N1 with the H275Y mutation prevailed

The persistence rates for cough were 7.1% (1/14), 46.2% (6/13), and 16.7% (5/30), respectively ($P = 0.033$ between 2007–2008 and 2008–2009), in children. The rates for adults were 20.0% (6/30), 42.1% (8/19), and 23.1% (12/52), respectively (NS among the three groups).

The persistence rates for the nasal symptoms of children were 7.1% (1/14), 61.5% (8/13), and 13.3% (4/30), respectively ($P = 0.004$ between 2007–2008 and 2008–2009, $P = 0.003$ between 2008–2009 and 2009–2010). The rates for adults were 10% (3/30), 21.1% (4/19), and 9.6% (5/52) in each season (NS among the three groups).

The persistence rates for sore throat (0–21.1%), myalgia or joint pain (0–6.7%), general fatigue (0–9.6%), and headache (0–3.3%) were low and without significance among the three groups for both children and adults.

Virus persistence after oseltamivir therapy

The interval between the first and second virus sampling was significantly longer in the 2008–2009 and 2009–2010 seasons (median of 5 days and 25th–75th percentile of 4–5 days in both seasons) than in the 2007–2008 season (median of 4 days and 25th–75th percentile of 4–5 days; $P = 0.014$ and $P = 0.002$, respectively), even though the study protocol was unchanged throughout the three seasons. No significant differences were found in the persistence rates of A(H1N1) virus after oseltamivir therapy among the three groups of adults 16 years and older (2007–2008, 10%; 2008–2009, 10.5%; and 2009–2010, 11.5%) (Fig. 2). In children 15 years and younger, there was also no statistically significant difference in the rates

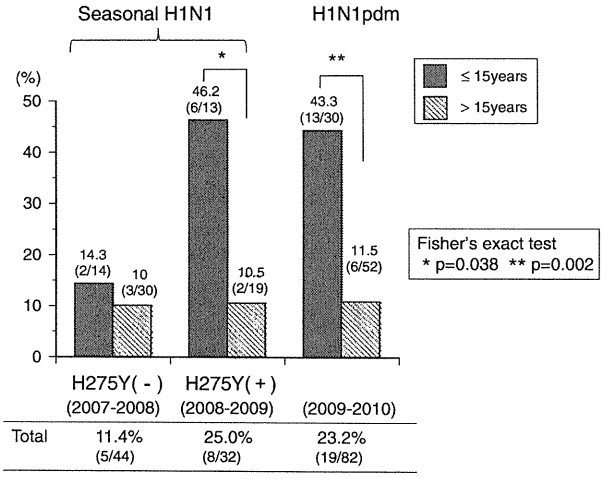

Fig. 2 Persistence rate of virus on the 4th–6th days after the start of therapy. The rate was significantly higher for children ≤15 years (*solid bars*) than for adults 15 years and older (*hatched bars*) in both 2008–2009 and 2009–2010 seasons

for the three seasons; however, the rate was higher in 2009–2010 and 2008–2009 than in 2007–2008 (43.3%, 46.2%, and 14.3%, respectively). The rates were significantly higher for children than for adults in both the 2008–2009 ($P = 0.038$) and 2009–2010 ($P = 0.002$) seasons (Fig. 2).

The persistence rates of A(H1N1) virus on day 4, day 5, and day 6 in all ages were 10.3%, 16.7%, and 0.0% in the 2007–2008 season, 33.3%, 26.7%, and 0.0% in the 2008–2009 season, and 34.5%, 14.6%, and 25.0% in the 2009–2010 season. No significant differences of the persistent rates were shown among days 4, 5, and 6 in each season.

For H1N1pdm in the 2009–2010 season, the viral persistence rate was significantly higher for patients aged 0–5 years (71.4%) than for those aged 16 years or older (11.5%; $P = 0.002$). It was also higher for patients aged 6–10 years (35.0%) than for patients 16 years or older (11.5%; $P = 0.037$) (Table 2).

H275Y mutation after therapy and IC_{50}

By NA sequence analysis, H275Y mutation was shown to have emerged after oseltamivir therapy in only two children with H1N1pdm in the 2009–2010 season. The frequency of emergence of H275Y mutation after oseltamivir therapy was 2.4% (2/82) for all patients and 6.7% (2/30) for children 15 years and younger. The frequency of patients in whom the virus persisted after oseltamivir therapy was 10.5% (2/19) of all patients and 15.4% (2/13) of children.

Table 2 H1N1pdm virus persistence rates in the 2009–2010 season by age cohort

Age	Persistence rates
0–5 years	71.4% (5/7)
6–10 years	35.0% (7/20)
11–15 years	33.3% (1/3)
16 years–	11.5% (6/52)

Fisher's exact test: * $P = 0.002$; ** $P = 0.037$

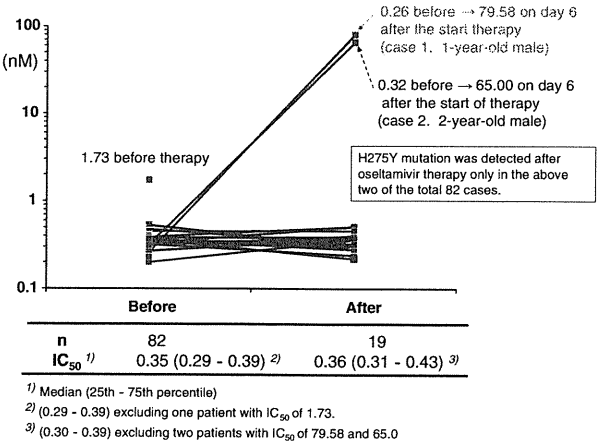

Fig. 3 IC_{50} for oseltamivir before and on days 4–6 after the start of therapy for patients with pandemic H1N1 in the 2009–2010 season. IC_{50} for oseltamivir was increased approximately 200- to 300 fold in two patients in whom the H275Y mutation emerged

The median IC_{50} was 0.35 nM (25th–75th percentile of 0.29–0.39 nM) before therapy and 0.36 nM (25th–75th percentile of 0.31–0.43 nM) after therapy (Fig. 3). The IC_{50} was increased 306 fold, from 0.26 to 79.58 nM (case 1, 2-year-old boy), and 203 fold, from 0.32 to 65.0 nM (case 2, 1-year-old boy) in two patients on day 6 after the start of oseltamivir therapy (Fig. 3). In both cases, H275Y mutation emerged after oseltamivir therapy. The highest body temperature of each day for case 1 was 38.3°C on day 1, 38.9°C on day 2, 37.6°C on day 3, 36.7°C on day 4, and 37.4°C on day 5; for case 2, highest body temperatures were 38.7°C on day 1, 36.6°C on day 2, 36.5°C on day 3, 36.6°C on day 4, and 36.6°C on day 5.

Discussion

Higher mortality rates (deaths per million population) by H1N1pdm 2009 were reported in many countries (Canada, 2.8; UK, 2.2; Mexico, 2.9; USA, 3.3; South Africa, 1.8; Argentina, 14.6; Australia, 8.6; Brazil, 7.0; Chile, 8.1; and New Zealand, 4.4) than in Japan, where the rate was extremely low (0.2) [20]. The wide use of commercial antigen detection kits by skilled physicians and the early start of anti-influenza drug therapy in Japan probably contributed to these results.

We previously reported in clinical and virological studies that oseltamivir was effective against seasonal influenza A(H3N2) and A(H1N1) until the 2007–2008 season, but that it was less effective for seasonal H1N1 with the H275Y mutation, especially in children [5–7, 10, 11]. In this study, no H275Y mutation was detected before treatment of H1N1pdm, and oseltamivir seemed to be effective for H1N1pdm in the 2009–2010 season, similar to seasonal H1N1 without the H275Y mutation (2007–2008 season) in terms of the rapid decline of fever and disappearance of other symptoms. However, viral persistence evaluated by virus culture was long for H1N1pdm, similar to seasonal H1N1 with H275Y mutation in the 2008–2009 season, especially in children 15 years and younger [10, 11]. We analyzed the viral persistence of patient cohorts 0–5, 6–10, 11–15, and 16 years of age and older in the 2009–2010 season, and the rate decreased with age.

In the 2008–2009 season, viral persistence was long because of reduced effectiveness of oseltamivir to the H275Y mutated virus [10]. However, the sensitivity of the virus to oseltamivir in the 2009–2010 season as evaluated by IC_{50} was quite comparable to that of seasonal H1N1 without H275Y mutation [10]. A long virus shedding period has also been reported, by RT-PCR, for young H1N1pdm patients [21–23]. The reason for the long virus persistence, irrespective of low IC_{50} of oseltamivir to H1N1pdm, is not clear. One possible explanation is that the long virus shedding period in H1N1pdm without H275Y mutation may be related to a low level of acquired immunity to a newly emergent influenza virus. Exposure to the seasonal H1N1 virus, which has similar immunological characteristics to H1N1pdm, may give some protection to the infected patients through cross-reactivity [24]. The low prevalence of H1N1pdm for persons more than 50 years old [25] and the excellent elevation of antibody titer by a single vaccination for H1N1pdm [26] in the 2009–2010 season seem to support this hypothesis. It should be noted that seasonal H1N1 virus cleared relatively early, even in children less than 16 years of age treated with oseltamivir. The long virus shedding after treatment with oseltamivir in young patients may be a characteristic of the H1N1pdm virus.

For H1N1pdm, the pre-therapy rate of H275Y mutation was low in this study (0%) similar to the other reports of the 2009–2010 season [13, 14]; however, the rate of this mutation after oseltamivir therapy has not been clearly studied, especially in outpatient clinics. In this study, H275Y mutation and 200- to 300-fold increases of IC_{50} were found in two children (2.4% of all subjects; 6.7% of children) after oseltamivir therapy. The H275Y mutation in our study may have been selected under oseltamivir pressure. The two patients did not show an especially prominent prolongation of fever, until day 4, and were cured without complication. No emergence of H275Y mutation after therapy was found for the adult outpatients of this study, and no E119V or N295S mutation reported to be related to oseltamivir resistance was detected [27]. However, it is important to pay careful attention to the appearance of H275Y mutation during or after either oseltamivir or peramivir therapy for patients with H1N1pdm in addition to the community-acquired H275Y mutation detected before therapy [16, 28].

In conclusion, oseltamivir was effective for fever and other clinical symptoms; however, viral persistence was longer than expected in children with H1N1pdm influenza in the 2009–2010 season. The frequency of H275Y mutation of H1N1pdm was low (2.4%) in this study of outpatients undergoing oseltamivir therapy.

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綜説

インフルエンザ感染と心筋炎

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総説

インフルエンザ感染と心筋炎*

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はじめに

2009年，40年ぶりに新型インフルエンザA(H1N1)pdmによるパンデミック(世界的大流行)が発生した[1,2]．本稿ではこのパンデミックを振り返るとともに，重篤な合併症であるインフルエンザ心筋炎について解説する．

インフルエンザウイルス感染の歴史

インフルエンザウイルスは直径80～120 nmのマイナス一本鎖のRNAウイルス(図1)でA，B，Cの3つの型がある．A型インフルエンザのウイルス粒子の表面には赤血球凝集素(HA)とノイラミニダーゼ(NA)という糖蛋白があり，HAに16種類，NAに9種類の亜型があるため計144種類の亜型が存在する．A型のみがパンデミックを起こすのは，その抗原性の違いにより多くの人が基礎免疫を持たないためである．2009年のパンデミック発生まではAソ連型(H1N1)とA香港型(H3N2)，およびB型が流行株であり，ワクチンもこれらに対する3価ワクチンが接種されてきた．これまでにヒトで流行が認められたのはH1N1とH3N2および約50年周期で流行したH2N2(アジア風邪型)のみである．パンデミック

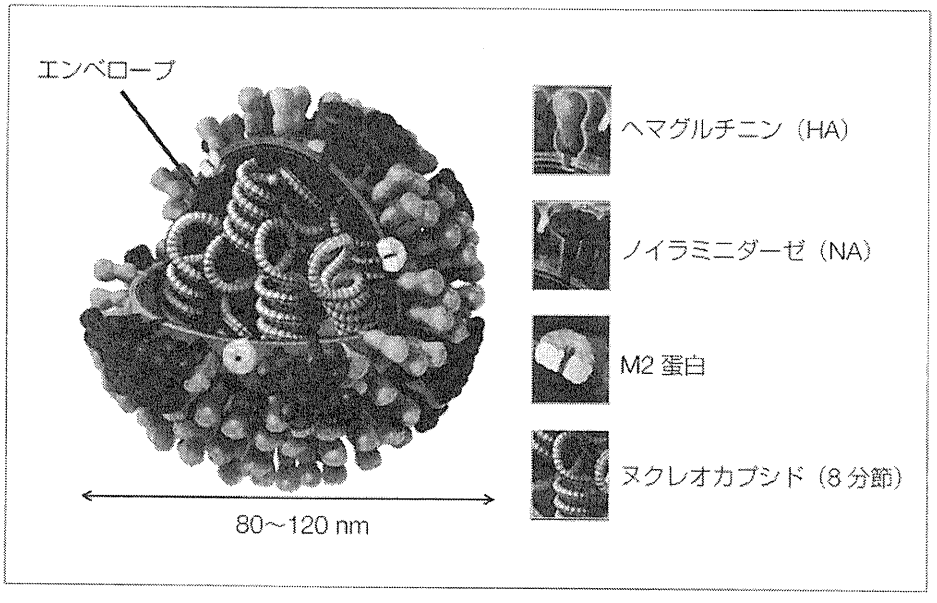

図1　インフルエンザウイルスの構造
ウイルスエンベロープの表面にはヘマグルチニン(HA)とノイラミニダーゼ(NA)の2種類の糖蛋白を有する．エンベロープの内部には8本の分節に分かれたゲノムがヌクレオカプシドとして存在し，それぞれのヌクレオカプシドにはRNA依存RNAポリメラーゼ複合体が結合している．
(CDCホームページより改変引用)

* Influenza Infection and Myocarditis Associated with Influenza

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前は高病原性のトリインフルエンザH5N1型あるいはH2N2型がパンデミックの原因ウイルスと想定され，高病原性を想定した対策が行政主導で策定された．しかし，今回のパンデミックはAソ連型と同じH1N1により発生し，高病原性を想定した対策とのずれのために対応現場では混乱を生じた．

今回の新型インフルエンザA(H1N1)pdmのウイルス学的特徴

今回，インフルエンザA(H1N1)pdmがAソ連型と同じH1N1であるにもかかわらずパンデミックが生じた理由は，A(H1N1)pdmとAソ連型の間に抗原性にかなりの違いがあったためと考えられている[3]．インフルエンザA(H1N1)pdmはかなり前に流行したと思われるヒト，トリ，ブタの遺伝子が交雑(リアソートメント)したウイルスとブタ由来のウイルスとが再び遺伝子の交雑を起こしたウイルスで，トリプルリアソータントと呼ばれている．NAとM蛋白の分節はユーラシアで流行のトリインフルエンザウイルスがブタに適応したものだが，これがスペイン風邪由来のものである．ブタではインフルエンザウイルスの遺伝子交雑が起こりやすく，今回の新型インフルエンザA(H1N1)pdm発生に重要な役割を果たしたことが明らかとなった．また，スペイン風邪のウイルスと比較すると，Aソ連型H1N1はヒトに感染を繰り返す過程において変異が多く起こりスペイン風邪のウイルスと抗原性に大きな相違があるのに対し，今回のインフルエンザA(H1N1)pdmでは変異が少なく，ブタに感染するウイルスとヒトで流行するウイルスとで変異の頻度が異なると考えられる．インフルエンザA(H1N1)pdmに対して高齢者が免疫を有していたとされ[4]，実際に発症者も少なかったが，その理由はウイルス変異による抗原性の差の大小に起因していると考えられる．こうしたウイルスの相違が病像に与える影響についてだが，動物感染モデルで今回の新型A(H1N1)は季節性Aソ連型(H1N1)より肺炎を起こしやすいと報告されている[4]．

今回の新型インフルエンザA(H1N1)pdmの疫学と臨床像

2009年春，新型インフルエンザA(H1N1)pdmが北米で発生，全大陸に感染が拡がりWHOはパンデミックと判断した[1,2]．2009年7月27日から2010年3月23日までの新型インフルエンザによる受診者数は約2,061万人と推計されるが，この数はインフルエンザ様症状を呈し，かつ医療機関を受診した患者数であり，医療機関を受診せずに自宅療養をした人や不顕性感染を含んでいない[5]．これは過去10年間でインフルエンザ(季節性インフルエンザ)の流行が最大であった2004年から2005年のシーズンの報告患者数148万人(推計1,770万人)を超えたが，ワクチンが間に合わなかったにもかかわらず，ピークの高さは季節性インフルエンザのそれを下回った．これには医療機関の対応，マスクの使用や手洗いの励行，学校閉鎖などの介入など各種対策が影響したと考えられる．また，これらの対策により患者数のピークが抑制されたことで，医療の現場が破綻せず対応を行えたと考えられる．発症者の総数の抑制は行えなくとも，ピークを抑制し，医療の現場が破綻しなかったことは重症例の救命率向上に寄与したと考えられる．

インフルエンザA(H1N1)pdmの症状は，比較的急速に出現する悪寒，発熱，頭痛，全身倦怠感，筋肉痛，咽頭痛，鼻汁，咳，下痢など季節性インフルエンザとほぼ同様であった．発症者の年齢分布は5～14歳に多く，中高年では少なかった．インフルエンザA(H1N1)pdmにより約18,000人が入院し，その約80%は小児であった．季節性インフルエンザでは高齢者の二次性肺炎が多いが，インフルエンザA(H1N1)pdmでは，若い年代でのウイルス性肺炎が多くみられた．肺炎の小児における多発は季節性と大きく異なる点であり，約10,000人が肺炎で入院したと推定されている．また，脳症の発症率はあまり季節性と差はなかったが，発症者の年齢が季節性に比して高めであったことが報告されている．インフルエンザは呼吸器がその感染の主座であり，今回のインフルエンザA(H1N1)pdmも基礎疾患として気管