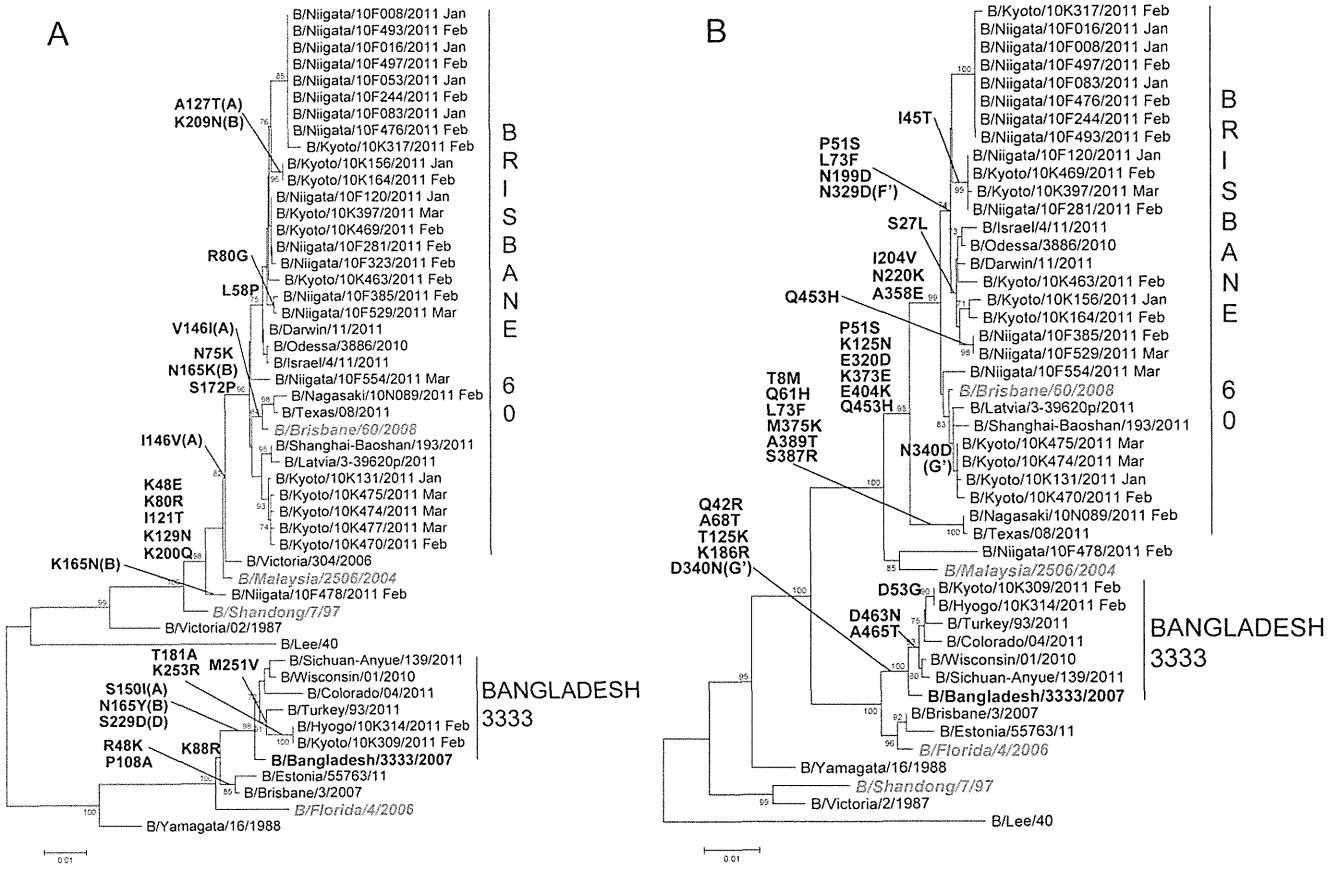

Figure 7. Phylogenetic analysis of the A) HA1 fragment of hemagglutinin, HA gene (885nt) and B) neuraminidase, NA gene (1,404nt) of influenza B viruses. Trees were constructed using the Neighbor-Joining method. Numbers at the nodes indicate confidence levels of bootstrap analysis with 1,000 replicates as percentage value. Amino acid substitutions that characterized a particular branch are indicated on the left side node. Vaccine strains are italicized and in red. Reference strains are boldfaced.
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The amino acid change S143G, which is found in 29/55 (52.7%) of viruses analyzed, is located next to the antigenic site Ca. In a previous report [29], two (2) viruses with the S143G substitution were observed to have reduced antigenicity against the A/California/07/2009 vaccine virus in the HI test. However, selected A(H1N1)pdm09 viruses with the S143G mutation from our study did not show a reduction in the HI titer when compared with the vaccine strain. These mutations in the HA did not contribute to a change in antigenicity of the A(H1N1)pdm09 viruses in our study.

The amino acid substitutions HA-D222G/N in A(H1N1)pdm09 viruses were previously associated with severe infection [15,16]. These mutations were not detected among the 81 isolates analyzed from the 2009–2010 season. Instead, a D222E substitution was found in 6 isolates representing 1.0% of viruses from the 2009–2010 period. This mutation was observed to be not associated with severe infection [16]. In the 2010–2011 season, the D222E mutation did not persist and all viruses had the wild-type genotype. The D222G/N mutations were not found in viruses in this season.

A new amino acid mutation in the NA of A(H1N1)pdm09 viruses associated with reduced susceptibility to neuraminidase inhibitors were recently reported. A mild reduction in oseltamivir and zanamivir susceptibility was observed in viruses from Oceania and Southeast Asia with the S247N (serine to asparagine) mutation [30]. We have not found this mutation in the viruses we analyzed.

Characteristics of A(H3N2) Viruses

Our results showed the prevalence of amantadine-resistant A(H3N2) viruses is high (99%) in the 2010–2011 season. The trend of high prevalence of amantadine resistance among A(H3N2) viruses was observed in Japan after the 2005–2006 season in Japan, reaching 100% prevalence in the 2008–2009 season [8,31]. However, in the post- pandemic (2010–2011) season, we detected 5 A(H3N2) viruses (1%) with the amantadine-susceptible S31 genotype in M2. Additional studies are needed to fully elucidate the genetic evolution of A(H3N2) viruses in relation to drug resistance or susceptibility.

Genetic analysis showed there were two groups of A(H3N2) viruses detected in the 2010–2011 season, A/Perth/16/2009-like and A/Victoria/208/2009-like [32]. However, A/Perth/16/2009-like viruses were mainly detected in prefectures where A(H3N2) viruses predominated in the beginning of the 2010–2011 season in December (Hokkaido and Nagasaki), while A/Victoria/208/2009-like viruses were detected in prefectures where A(H1N1)pdm09 predominated and started to circulate only in January (Hyogo and Osaka). These findings suggest that the timing and circulation patterns of the two clades were different and that these viruses may have been transmitted to Japan from other countries at different timing and routes.

Our sequence analysis showed additional mutations in the HA of 2010–2011 viruses from previously circulating strains. Several amino acid substitutions in the putative antigenic sites in HA and

NA were found in Perth16 and Victoria208 isolates when compared with the vaccine strain, A/Perth/16/2009. The mutations in the HA may have contributed to the reduced antigenicity against the vaccine strain of some Vic208 clade viruses [29]. Only Perth16 clade viruses underwent HI testing in our study and these viruses did not show a reduction in titer against the vaccine strain. Although all viruses tested were A/Perth/16/2009-like, these viruses have similar antigenicities with A/Victoria/208/2009-like viruses as previously reported [33].

Characteristics of Influenza B Viruses

There was cocirculation of Victoria-lineage and Yamagata lineage influenza B viruses in the 2010–2011 season. Most of the influenza viruses isolated belong to the Victoria lineage in the HA and were similar to the vaccine strain, B/Brisbane/60/2008. These reassortant Victoria lineage viruses that had a Yamagata lineage NA have been stably circulating worldwide since its emergence in 2002 [34]. The evolution of the NA is parallel with that of the HA as evidenced in the similar clustering of viruses in the phylogenies although the NA belongs to the same Yamagata lineage.

In summary, all three predominantly circulating viruses in the 2010–2011 season demonstrated genetic variability from the previously circulating strains but were closely related to the 3 strains recommended by the WHO to be included in the vaccine for the 2011–2012 influenza season in the northern hemisphere: an A/California/7/2009-like virus, an A/Perth/16/2009-like virus and a B/Brisbane/60/2008-like virus [35]. In addition, only a small percentage of influenza A viruses tested was resistant to oseltamivir but almost all were resistant to amantadine.

It remains to be seen whether the pandemic A(H1N1) 2009 virus will follow the path of other pandemic viruses such as that of the Asian influenza A(H2N2) virus which survived shortly in the human population and disappeared 11 years after its emergence [36] or that of the Hong Kong influenza A(H3N2) virus which 43 years later still remains an important cause of influenza illness in humans [36].

Materials and Methods

Study Design

Eligible patients to this study were those who visited outpatient clinics presented with influenza-like illness symptoms (fever of $\geq$37.5°C, cough and/or sore throat) and had not been treated with amantadine, oseltamivir or zanamivir within the previous four weeks. The study period was between July 30, 2009 and March 22, 2011. The Niigata University Division of International Health (Public Health) supplied viral transport media to clinicians participating in the Japanese Influenza Collaborative Study Group (18 clinics in eight Prefectures in Japan: Hokkaido, Fukushima, Gunma, Niigata, Kyoto, Hyogo, Osaka and Nagasaki Prefectures). Clinicians performed an influenza rapid diagnostic test, mainly by using the Quick-Ex Flu kit (Denka Seiken, Co. Ltd., Tokyo, Japan), on the first respiratory specimen, and collected a second respiratory specimen regardless of the rapid test results. The samples were stored in viral transport media for $\leq$72 hours at 4°C before shipment to our laboratory. Informed written consent was obtained from the patient or the patient's guardian prior to specimen collection. The study was approved by the medical faculty ethics committee of the Niigata University Graduate School of Medical and Dental Sciences.

Virus Isolation and Characterization

About 100 µl of nasopharyngeal sample was inoculated onto Madin-Darby canine kidney (MDCK) cells. MDCK cell lines were kindly provided by Dr. Hidekazu Nishimura of Virus Center, Sendai National Medical Center, Miyagi Prefecture, Japan and were maintained as previously described [37]. Cultures were monitored for cytopathic effect (CPE) for 3–7 days. All isolates were typed and subtyped by cycling probe real-time PCR assays. Selected influenza isolates underwent a hemagglutination inhibition (HI) assay using guinea pig red blood cells (Toyo Bio, Tokyo, Japan) and commercially available influenza vaccine strain antisera: A/California/7/2009 (pandemic H1N1), A/Brisbane/59/2007 (seasonal H1N1), A/Victoria/210/2009 (H3N2), B/Brisbane/60/2008 (influenza B, Victoria) and B/Florida/4/2006 (influenza B, Yamagata) (Denka Seiken Co., Ltd., Tokyo, Japan).

RNA Extraction and cDNA Synthesis

Total RNA was extracted from 100 µl of clinical specimens and from 100 µL of viral culture using Extragen II kit (Kainos, Tokyo, Japan) following manufacturer's instructions. First-strand cDNA synthesis was performed using influenza A and B universal primers [37,38].

Real-time PCR Genotyping of Drug-resistant Strains

Cycling probe real-time PCR screening for mutations in M2 and NA genes that confer resistance to amantadine and oseltamivir, respectively, was performed on all 1,540 influenza A virus isolates (2009–2010 and 2010–2011) using fluorescent-labeled chimeric RNA-DNA probes and RNaseH (TaKaRa Bio Inc., Ohtsu, Japan). This assay utilizes a single nucleotide polymorphism (SNP) which can simultaneously detect amantadine-sensitive (S31) and amantadine-resistant (S31N) viruses as previously described [39]. Similarly, another single nucleotide polymorphism which can distinguish between oseltamivir-sensitive (H275) and oseltamivir-resistant (H275Y) viruses was used as previously described [40]. SNP typing was performed using Thermal Cycler Dice Real Time PCR System TP800 (TaKaRa Bio Inc., Ohtsu, Japan).

Cycling Probe Real-time PCR Method for Influenza B

Samples that were negative for the screening methods for A(H1N1)pdm09 and A(H3N2) were tested using a cycling probe real-time PCR method that can distinguish Victoria-lineage and Yamagata-lineage influenza B viruses according to the HA gene sequence.

For the influenza B cycling probe real time PCR reaction, a commercially available cycling probe real time PCR kit, TaKaRa CycleavePCR®Core Kit (TaKaRa Bio Inc., Ohtsu, Japan) was used. The PCR reaction was prepared according to the manufacturer's instructions and was supplemented with 5 pmol of each primer (a forward primer, a Victoria lineage-specific reverse primer and a Yamagata lineage-specific reverse primer), 2.5 pmol of the carboxyfluorescein (FAM)-labeled probe (Victoria HA gene-specific), 2.5 pmol of the carboxy-X-rhodamine (ROX)-labeled probe (Yamagata HA gene-specific), and 1 µL of viral cDNA in a 25 µl reaction volume. PCR amplification and fluorescence detection were performed on Thermal Cycler Dice Real Time PCR System TP800 (TaKaRa Bio Inc., Ohtsu, Japan). Cycling conditions were as follows: denaturation at 95°C for 10 seconds followed by 40 cycles of denaturation at 95°C for 5 seconds, primer annealing at 57°C for 10 seconds, and extension and subsequent detection of fluorescence at 72°C for 15 seconds (primers and probes information are available on request).

DNA Sequencing and Phylogenetic Analysis

The HA and NA genes of 136 A(H1N1)pdm09, 71 A(H3N2) and 29 influenza B viruses, as well as the M gene of 6 A(H1N1)pdm09 viruses from the 2009–2010 season, were amplified using gene-specific primers. PCR products were purified using MSBP Spin PCRapace kit (Invitek GmbH, Berlin, Germany) and directly sequenced. Sequencing reactions were carried out using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Carlsbad, USA) and sequencing products were run on an ABI Prism 3100 Genetic Analyzer. Sequences were assembled using Lasergene SeqMan Pro package version 7.2.1 (DNASTAR, Madison, USA) and assembled sequences were edited using BioEdit software (http://www.mbio.ncsu.edu/BioEdit/). Phylogenetic analysis was performed using MEGA 4.0 software [41] (Molecular Evolutionary Genetics Analysis). Best-fitting trees for the HA and NA genes were constructed by the Neighbor-Joining method [42] with the maximum composite likelihood model [43] and bootstrap analysis of 1,000 replicates. Deduced amino acid sequences were analyzed and changes in the antigenic sites were compared among isolates with the respective vaccine strains: A/California/7/2009 (H1N1pdm); A/Perth/16/2009 (H3N2); and B/Brisbane/60/2008 (influenza B). In this study, all HA amino acid residues are numbered without the signal peptide sequence. HA and NA numbering are based on the respective type and subtype.

GenBank accession numbers of the HA and NA sequences of Japanese A(H1N1)pdm09 strains from the 2009–2010 season are CY066017–CY066182 and from the 2010–2011 season are JN790349–JN790440. The accession numbers for the HA and NA sequences of A(H3N2) strains from the 2010–2011 season are JN790441–JN790581, and for the influenza B strains are JN790295–JN790348. Vaccine strain sequences and supplemental sequences used for the phylogenetic trees were obtained from the GISAID EpiFlu^{TM} Database (www.gisaid.org) and from GenBank (www.ncbi.nlm.nih.gov/genbank).

Antigenic Site Mapping of HA and NA

HA and NA amino acid sequences of influenza virus isolates in Japan were compared with the vaccine strains. Identified amino acid substitutions were mapped to reported HA antigenic sites [14,18,20] and NA antigenic sites [17,19]. Crystal structures of HA (PDB entry 3LZG for H1, 1MQL for H3, and 2RFT for type B) and NA (PDB entry 3NSS for N1, 1IVG for N2, and 1INF for type B) were downloaded from Protein Data Bank (RCSB PDB, http://www.pdb.org) [44]. Molecular models were analyzed using the PyMol software v1.3 (http://www.pymol.org).

Supporting Information

Figure S1 Phylogenetic analysis of the M gene of amantadine-sensitive A(H1N1)pdm09 viruses. Trees were constructed using the Neighbor-Joining method. Numbers at the nodes indicate confidence levels of bootstrap analysis with 1,000 replicates as percentage value. Human A(H1N1)pdm09 strains from the present study are in bold. Amantadine-sensitive strains (AmS) are indicated with filled triangles (▲). Other viruses included in the analysis were based on the study by Vijaykrishna D *et al.* (2010) [45] and the sequences were obtained from GenBank. The Japanese swine sequences were also obtained from GenBank.
(TIF)

Table S1 Hemagglutination inhibition (HI) titer of selected A(H1N1)pdm09 viruses from the 2010–2011 season.
(XLS)

Table S2 Hemagglutination inhibition (HI) titer of selected A(H3N2) viruses from the 2010–2011 season.
(XLS)

Table S3 Hemagglutination inhibition (HI) titer of selected influenza B viruses from the 2010–2011 season.
(XLS)

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Author Contributions

Conceived and designed the experiments: RS HS ICD CD Y. Suzuki. Performed the experiments: ICD CD TB Y. Suzuki HK. Analyzed the data: ICD CD TB Y. Suzuki RS HS. Contributed reagents/materials/analysis tools: Y. Shobugawa. Wrote the paper: ICD RS HS.

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ORIGINAL ARTICLE

Clinical effectiveness of neuraminidase inhibitors—oseltamivir, zanamivir, laninamivir, and peramivir—for treatment of influenza A(H3N2) and A(H1N1)pdm09 infection: an observational study in the 2010–2011 influenza season in Japan

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Abstract The clinical effectiveness of the newly released neuraminidase inhibitors (NAIs) laninamivir and peramivir has not been sufficiently evaluated in influenza-infected patients in clinical and practical settings. In this study, we analyzed the clinical data of 211 patients infected with influenza A virus subtype H3N2 (A(H3N2)) and 45 patients infected with influenza A virus subtype H1N1pdm (A(H1N1)pdm09) who received the NAIs oseltamivir, zanamivir, laninamivir, or peramivir during the 2010-2011 influenza season. The duration of fever from the first dose of the NAI to fever alleviation to <37.5 °C was evaluated as an indicator of the clinical effectiveness of the NAIs in the influenza-infected patients. For the A(H3N2)-infected patients, Kaplan–Meier analysis showed the peramivir treatment group had the fastest time of fever alleviation to <37.5 °C (median 17.0 h, 95 % confidence interval [CI] 7.2–26.8 h) of the four treatment groups. No significant difference was found in the time to fever alleviation among the other antivirals, oseltamivir, zanamivir, and laninamivir. Results of multivariate analysis, using a Cox proportional-hazards model (hazard ratio 3.321) adjusted for the factors age, sex, body weight, vaccination status, time from onset to the clinic visit, and body temperature showed significantly faster fever alleviation in the peramivir treatment group compared with the oseltamivir treatment group. For the A(H1N1)pdm09-infected patients, only the oseltamivir and zanamivir treatment groups were compared, and no significant difference in time to alleviation of fever was observed between the two groups. Based on a cycling probe real-time polymerase chain reaction (PCR) assay, none of the A(H1N1)pdm09 strains in this study had the H275Y mutation conferring oseltamivir resistance. Further evaluation of the clinical effectiveness of the newly released NAIs for influenza-infected patients, including those infected with A(H1N1)pdm09, is needed.

Keywords Influenza virus · Neuraminidase inhibitors · Fever alleviation · Clinical effectiveness · Observational study

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Introduction

Yearly influenza epidemics have raised medical and social concerns because they are associated with considerable morbidity and mortality [1]. Two countermeasures, vaccination and antivirals, are available to control human influenza infections [2]. Although vaccinations may play a critical role in influenza prophylaxis, they are insufficient in a pandemic because of the time required to produce a new vaccine. Therefore, antivirals are an important tool to

mitigate the effects of an influenza pandemic. Neuraminidase inhibitors (NAIs), rather than M2 inhibitors, have been preferred for the treatment of influenza infection, owing to the continuing high prevalence of influenza A virus subtype H1N1pdm ((H1N1)pdm09) and influenza A virus subtype H3N2 (A(H3N2)) resistance to the latter drugs [3, 4]. In this situation, the licensing of new NAIs other than oseltamivir and zanamivir has important implications as an alternative option for the treatment of influenza infection [5]. In 2010, two new NAIs, laninamivir and peramivir, were approved for clinical use for the treatment of influenza A and B infection in Japan, as one of the earliest countries to license these compounds. Clinicians have limited experience of using these drugs other than information from clinical trials [6–10]. In the present study, we report on the effectiveness of NAIs, including early data for the two newly released drugs, peramivir and laninamivir, by observing fever alleviation in influenza-infected patients in the 2010-2011 season.

Patients and methods

Study procedures

This study was conducted in the 2010-2011 winter season from December 27, 2010, to March 29, 2011, in Japan. Two outpatient clinics in two prefectures, Niigata and Gunma, participated in this study. As a rule, randomly selected patients with influenza-like-illness (ILI) who visited the clinics were enrolled. ILI was defined as sudden onset of fever (≥37.5 °C) and respiratory symptoms, as well as headache, arthralgia, or myalgia. Clinicians used influenza rapid diagnostic test kits licensed in Japan in the patients who had ILI symptoms to screen for influenza A or B infection. Of the commercially available antigen detection kits based on immunochromatography, Quick Ex flu (Denka Seiken, Tokyo, Japan), ESPLINE Influenza A&B-N (Fujirebio, Tokyo, Japan), and Check FluA·B (Alfresa Pharma, Osaka, Japan) were mainly used. If the rapid test was positive, patients were given one of four NAIs, oseltamivir, zanamivir, laninamivir, or peramivir, according to the advice of clinicians and the preference of the patients or their guardians. Upon enrollment in this study, written informed consent was obtained from the patient or their guardian for the collection of clinical information and specimens for virological investigations.

Oseltamivir was prescribed to be taken orally twice daily for 5 days (150 mg per day for adults and for children who weighed ≥37.5 kg, and 4 mg/kg for children who weighed <37.5 kg), and zanamivir inhalation was prescribed to be used twice daily for 5 days (20 mg per day for adults and for children ≥5 years old). Laninamivir was prescribed as a single inhalation (40 mg for adults and for children ≥10 years old, or 20 mg for children <10 years old as a treatment course), and peramivir was administered as a single intravenous drip infusion at the outpatient clinic (300 mg for an adult without complications, 600 mg for adult patients at high risk of developing complications, and 10 mg/kg for children, with a maximum dose of 600 mg as a treatment course). All dosages followed the advisories issued by the Ministry of Health, Labor, and Welfare of Japan. Single use of antipyretics was allowed when a patient had a fever of ≥38.5 °C. Clinicians were permitted to prescribe other drugs such as cough suppressants and expectorants depending on the patient's complaints.

Age, sex, vaccination status, body weight, and body temperature were recorded for all eligible patients. The date and time of onset of the fever, and the dates of clinic visits were recorded by the doctors. Time of onset was defined when a patient had a fever of more than 37.5 °C before the clinic visit. Time of administration of the first dose of antiviral was defined as the time of the clinic visit, because clinicians administered the drug at the clinics (peramivir), or instructed the patients to take the drug as soon as they reached home (oseltamivir and zanamivir) or at the pharmacy (laninamivir). Patients were asked to measure and record axillary body temperature at least three times per day (9 a.m., 12 p.m., and 8 p.m.) at home. Then they sent the record of information on daily body temperature by mail, or brought the record directly to the clinic after alleviation of the fever. The time at which the body temperature dropped below 37.5 °C without recurrence was defined as the time at which the patient became afebrile. This study was approved by the Medical Faculty Ethics Committee of the Niigata University Graduate School of Medical and Dental Sciences.

Virological investigation

Specimens from throat swabs, nasal swabs, or nasal aspirates taken at the time of the clinic visits were submitted for virus isolation.

Clinical samples were inoculated on Madin-Darby canine kidney (MDCK) cells for virus isolation. The typing and subtyping of influenza virus isolates into A(H1N1)pdm09, A(H3N2), and type B was performed by hemagglutinin inhibition test or a real-time polymerase chain reaction (PCR) method [11]. Analysis of oseltamivir resistance in A(H1N1)pdm09 samples was performed by using real-time PCR with a cycling probe assay which can specifically detect the H275Y substitution in the NA gene conferring oseltamivir resistance [12].

Statistical analysis

For estimating fever duration after administration of the first dose of the antiviral in the patients who received oseltamivir, zanamivir, laninamivir, or peramivir, Kaplan–Meier analyses were applied and the log rank test was used for comparisons of estimated fever duration among the treatment groups. For multivariate analysis, we used a Cox proportional-hazards model to evaluate the duration of fever from the first dose of the NAI to fever alleviation <37.5 °C. We set the duration of fever (hours) as a dependent variable, and set the following factors as independent variables: the type of treatment (oseltamivir, zanamivir, laninamivir, or peramivir), age, sex, body weight, vaccination status, time from onset to the clinic visit, and body temperature at the clinic visit. All statistical analyses were performed using SPSS software, version 19.0 (IBM, Armonk, NY, USA). P values of <0.05 were employed to define statistical significance.

Results

Patient characteristics

A total of 681 patients in the two clinics were enrolled in the study during the 2010–2011 season. After excluding patients for whom there was incomplete clinical information, data for analysis were obtained from 263 eligible patients; that is, 211 with A(H3N2) virus infection, 45 with A(H1N1)pdm09 infection, and 7 with influenza virus type B infection. The demographic characteristics of the patients are shown in Table 1. Of the 211 patients infected with A(H3N2) virus, 104 were treated with oseltamivir, 94 were treated with zanamivir, 9 were treated with laninamivir, and 4 were treated with peramivir. Of the 45 patients infected with A(H1N1)pdm09 virus, 15 were treated with oseltamivir and 30 were treated with zanamivir. None of the patients who were infected with A(H1N1)pdm09 virus were treated with laninamivir or peramivir. Further evaluation was done only for patients infected with the A(H3N2) and A(H1N1)pdm09 viruses, because the sample size for the influenza B virus-infected patients was too small ($N = 7$) to analyze in detail. In the patients infected with A(H3N2) virus, the average age in the oseltamivir treatment group was significantly lower than that in the other treatment groups. The time from the onset of fever to the first clinic visit was shorter in the peramivir treatment group than in the other groups. The average body temperature during the clinic visit was significantly higher in the oseltamivir group than that in the zanamivir group.

Regarding the patients infected with A(H1N1)pdm09 virus, the average age in the oseltamivir treatment group was significantly lower than that in the zanamivir treatment group. In both the A(H3N2) and A(H1N1)pdm09 virus-infected patients, no other statistically significant differences in baseline status items such as sex, time to the first clinic visit, or vaccination status were detected among the patients who received oseltamivir, zanamivir, laninamivir, and peramivir (Table 1).

In the genetic analysis, no H275Y substitution in the NA gene conferring oseltamivir resistance was detected in any of the 45 A(H1N1)pdm09 strains.

Duration of fever after administration of the first dose of antiviral therapy

For the A(H3N2)-infected patients, the duration of fever was evaluated in the four treatment groups–oseltamivir, zanamivir, laninamivir, and peramivir. Kaplan–Meier analysis showed that the time to alleviation of fever in the peramivir treatment group (17.0 h, 95 % CI 7.2–26.8 h) was significantly shorter than that in the oseltamivir treatment group ($P = 0.044$, Fig. 1). No significant difference was found in times to fever alleviation among the other three treatment groups–oseltamivir, zanamivir, and laninamivir.

In the influenza A(H1N1)pdm09-infected patients, only oseltamivir and zanamivir treatment groups were compared, because there were no groups treated with the new drugs. In the Kaplan–Meier analysis, no significant difference was detected between the oseltamivir and zanamivir treatment groups in the time to alleviation of fever (Fig. 1).

In the A(H3N2)-infected patients, the Cox proportional-hazards model showed that the time to alleviation of fever was 3.3 times shorter in the peramivir treatment group than that in the oseltamivir treatment group, with an adjusted P value of 0.0303 (Table 2). No significant difference in the time to alleviation of fever was found in the zanamivir and laninamivir treatment groups when compared with the oseltamivir treatment group. In the A(H1N1)pdm09-infected patients, the Cox proportional-hazards model showed no statistically significant difference in the time to alleviation of fever between the oseltamivir and zanamivir treatment groups.

Discussion

In this study, we evaluated the clinical effectiveness of NAIs by analyzing the duration of fever after initiation of therapy in patients with influenza A infection treated with oseltamivir, zanamivir, laninamivir, or peramivir in Japan. We found no statistically significant difference between the oseltamivir treatment group and the zanamivir treatment

Table 1 Baseline demographic and clinical characteristics of patients infected with influenza A virus subtype H3N2 and influenza A virus subtype H1N1pdm in the 2010–2011 season

Subtype of influenza	Value			
	Oseltamivir treatment group	Zanamivir treatment group	Laninamivir treatment group	Peramivir treatment group
A(H3N2)				
n	104	94	9	4
Male/female	53/51	61/33	3/6	3/1
Age (years), mean ± SD	5.1 ± 2.5[a]	9.1 ± 2.0	10.2 ± 2.3	8.8 ± 3.9
Body weight (kg), mean ± SD	18.7 ± 11.0[b]	28.9 ± 8.5	32.6 ± 8.9	28.4 ± 14.5
Body temperature at the clinic visit (°C), mean ± SD	38.5 ± 0.8[c]	38.2 ± 0.7	38.4 ± 1.1	38.9 ± 0.8
Time from onset to the first clinic visit (h), mean ± SD	15.4 ± 10.1	15.0 ± 9.9	9.3 ± 12.1	3.0 ± 1.2
Vaccination status, vaccinated/unvaccinated	57/104 (54.8 %)	54/94 (57.4 %)	4/9 (44.4 %)	3/4 (75.0 %)
A(H1N1)pdm09				
n	15	30		
Male/female	7/8	17/13		
Age (years), mean ± SD	4.6 ± 2.0[d]	9.7 ± 2.9		
Body weight (kg), mean ± SD	17.0 ± 4.3[e]	30.7 ± 9.6		
Body temperature at the clinic visit (°C), mean ± SD	38.2 ± 0.7	38.4 ± 0.7		
Time from onset to the first clinic visit (h), mean ± SD	14.1 ± 6.3	13.2 ± 9.0		
Vaccination status, vaccinated/unvaccinated	11/15 (73.3 %)	16/30 (53.3 %)		

[a] The age of the oseltamivir group was significantly lower than that of the zanamivir group ($P < 0.001$), that of the laninamivir group ($P < 0.001$), and that of the peramivir group ($P = 0.012$)

[b] Body weight in the oseltamivir group was significantly lower than that of the zanamivir ($P < 0.001$) and laninamivir groups ($P = 0.001$)

[c] Body temperature in the oseltamivir group was significantly higher than that in the zanamivir group ($P = 0.046$)

[d] The age of the oseltamivir group was significantly lower than that of the zanamivir group ($P < 0.001$)

[e] The body weight in the oseltamivir group was significantly lower than that in the zanamivir group ($P < 0.001$)

group in the patients who were infected with either the A(H3N2) or A(H1N1)pdm09 viruses. Kawai et al. reported that oseltamivir was more effective than zanamivir in influenza A(H3N2)- or A(H1N1)pdm09-infected patients who were mostly adults [13, 14]. On the other hand, Sugaya et al. [15] showed that there was no significant difference in the effectiveness of oseltamivir and zanamivir against A(H3N2) in children. Our result may support the study of Sugaya et al. because most of the patients in our study were children.

We found that A(H3N2)-infected patients who underwent peramivir therapy showed faster alleviation of fever compared to that shown in A(H3N2)-infected patients treated with the other three antivirals. Even after adjustment by the factors age, sex, body weight, vaccination status, time from fever onset to the clinic visit, and body temperature at the clinic visit, patients who received peramivir still showed significantly faster reduction of fever compared with the findings in the other study groups.

In a previous study, Kohno et al. [6] showed the efficacy of peramivir therapy in adult patients with seasonal influenza virus infection (A(H1N1) and A(H3N2)) compared with results in a placebo group, and in a randomized control study [7], they demonstrated the non-inferiority of peramivir, compared with oseltamivir, in reducing the time to alleviate influenza symptoms in patients with seasonal influenza virus infection (A(H1N1)pdm09, A(H3N2), or influenza virus type B). Sugaya et al. [8] reported the efficacy of peramivir treatment in A(H1N1)pdm09-infected children. However, so far only a few studies have documented the efficacy of peramivir in patients infected with influenza virus; thus, the present study is still meaningful from the clinical point of view. In addition, at present, only in Japan peramivir has been approved for clinical use. Thus, it is important to document early experiences in its clinical use in Japan, as shown in our study.

In our study, the sample size for analysis was small, especially in the peramivir and laninamivir treatment

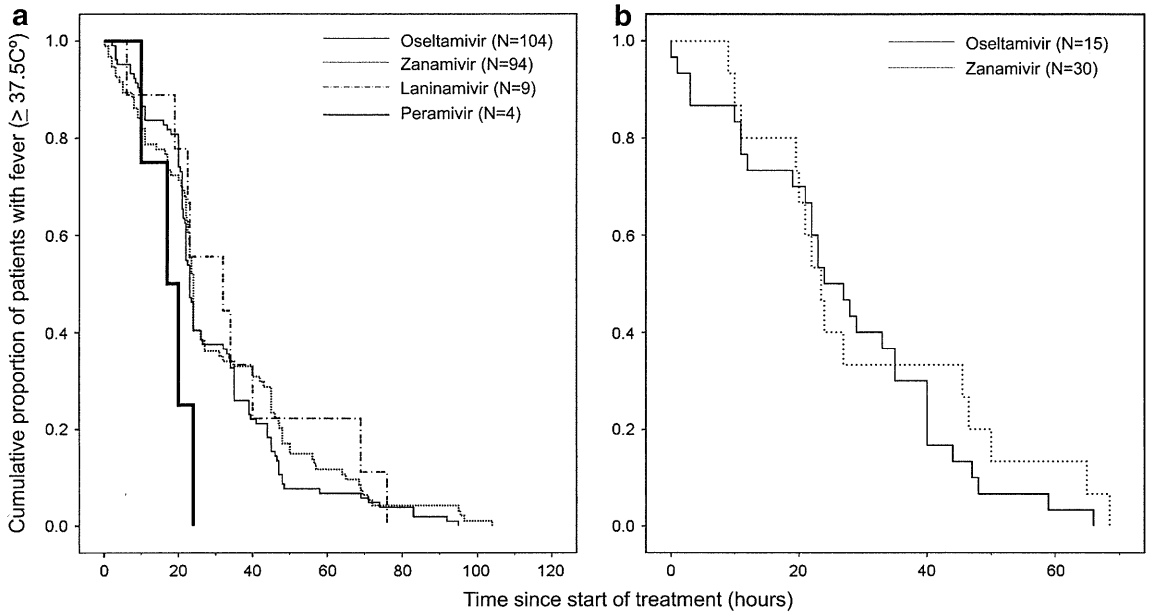

Fig. 1 Kaplan–Meier curves of time to return to body temperature <37.5 °C in the influenza A(H3N2)-infected patients (**a**), and the influenza A(H1N1)pdm09-infected patients (**b**)

Table 2 Time to alleviation of fever analyzed using the Cox proportional-hazards model

Population and treatment	*n*	Median duration to fever alleviation, h (95 % CI)	Hazard ratio (95 % CI)[a]	Adjusted *P* value
A(H3N2)				
Oseltamivir	104	23.0 (22.0–24.0)	Reference	
Zanamivir	94	24.0 (23.3–24.7)	0.854 (0.581–1.255)	0.4212
Laninamivir	9	32.0 (5.7–58.3)	0.880 (0.404–1.915)	0.7467
Peramivir	4	17.0 (7.2–26.8)	3.321 (1.121–9.836)	0.0303[b]
A(H1N1)pdm09				
Oseltamivir	15	23.5 (19.7–27.3)	Reference	
Zanamivir	30	24.0 (17.6–30.4)	1.065 (0.389–2.917)	0.9018

CI confidence interval

[a] Hazard ratios compared with the oseltamivir treatment group were estimated using Cox proportional-hazards modeling, adjusted for age, sex, body weight, vaccination status, time from onset to the clinic visit, and body temperature at the clinic visit

[b] The probability of faster alleviation of fever was significantly higher in the peramivir treatment group than in the oseltamivir group

groups. This limitation may affect the reliability of the study. Also, in the peramivir group, the time from fever onset to the clinic visit was shorter than that in the other groups. Perhaps the early visit to the clinic in the peramivir treatment group may have led to the shorter duration of fever. However, the duration of fever in the peramivir group was still significantly shorter than that in the other study groups when we compared fever duration using multivariate analysis adjusting for factors including time from fever onset to the clinic visit.

Another limitation of our study is that the antiviral drug prescribed depended on the discretion of clinicians or the preference of patients or their guardians. Laninamivir and peramivir were newly released in Japan in 2010. In addition, in most cases, oseltamivir was not advised for use by patients of 10–19 years of age in Japan because of reports of an association with neuropsychiatric symptoms in this age group [5]. Zanamivir is not recommended for patients with underlying respiratory disease or for children <5 years old. Therefore, the decision whether to administer one of the four drugs to patients was left mostly to the discretion of clinicians, who followed the Japanese guidelines outlined above and patient preference. This non-randomized manner of drug allocation may have caused selection bias in this observational study.

Peramivir is an anti-influenza drug that selectively inhibits the neuraminidase of human type A and type B influenza viruses. The most important characteristic of

peramivir is its rapid bioavailability when administered intravenously. Current antiviral treatments other than peramivir– such as oseltamivir, zanamivir, and laninamivir– are administered either orally or by inhalation. These routes may not provide rapid, reliable drug delivery in seriously ill patients. In view of the certainty of drug delivery with intravenous administration, peramivir may be administered as the first-line therapy, especially in patients who have high-risk factors for complications or those who cannot take drugs orally or inhale drugs.

In our study, in the patients with influenza A(H3N2) infection, no significant difference in duration of fever was shown in the laninamivir treatment group compared with the other study groups. Watanabe et al. [10] showed the non-inferiority of laninamivir, compared with oseltamivir, in patients with seasonal influenza (A(H1N1)pdm09 and A(H3N2)). Sugaya and Ohashi [9] showed more rapid alleviation of influenza illness in children infected with seasonal A(H1N1) who received laninamivir than in those who received oseltamivir. The reason for the slower alleviation of influenza illness in patients with seasonal A(H1N1) virus infection who received oseltamivir than in those who received laninamivir was possibly that almost all seasonal A(H1N1) viruses possessed the H275Y mutation, which conferred resistance to oseltamivir [16].

Inhaled laninamivir was developed in Japan and approved for use in our country in 2010. Laninamivir octanoate has been shown to have neuraminidase inhibitory activity against various influenza A and B viruses, including oseltamivir-resistant viruses [10]. The chemical structure of the active drug, laninamivir, is similar to that of zanamivir. The most important characteristic of laninamivir octanoate is its long-lasting antiviral activity, and because of this, laninamivir is administered in the form of a single inhalation on the first day of treatment, and it stays active in the respiratory tract for several days [17].

During the 2009 influenza pandemic, the use of oseltamivir increased dramatically, and this raised a concern about the emergence of oseltamivir resistance that could limit the effectiveness of NAIs. Fortunately, oseltamivir-resistant A(H1N1)pdm09 strains are still rare in the community [18, 19]. However, sporadic cases of oseltamivir-resistant A(H1N1)pdm09 infections were detected in Japan soon after community circulation of the A(H1N1)pdm09 virus was observed [20]; thus, the spread of drug-resistant viruses has become a major concern in the post-pandemic period [16, 21, 22]. We must always pay attention to appearance of resistant viruses possessing the H275Y mutation conferring oseltamivir and peramivir resistance that may reduce the clinical effectiveness of these drugs.

In this report, we demonstrated the clinical effectiveness of the new NAIs. Because the number of cases we studied was limited, further clinical study to investigate the effectiveness of new NAIs in influenza infections is important.

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Conflict of interest None.

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4種のノイラミニダーゼ阻害薬の使い方

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ノイラミニダーゼ阻害薬(NAI)は2010～2011年シーズンからラニナミビルを加え4剤時代となった．同シーズンの日本臨床内科医会研究グループの使用状況では，オセルタミビルは9歳以下，ザナミビルは10代，ペラミビルは20歳以上が各6割以上を占め，ラニナミビルは5歳以上の各年代で幅広く使用された．各薬剤の傾向として，解熱時間はA(H1N1) pdm09が最短で，以下A(H3N2)，Bの順であり，IC_{50}(NA活性の50%阻害濃度)もほぼ同様であった．重回帰分析で解熱時間などにNAIの種類は影響せず，(亜)型が有意に影響した．ラニナミビルのウイルス残存率は過去のオセルタミビルやザナミビルと遜色なかった．以上，NAIの有効性は薬剤間の差は少なく，投与経路・回数，耐性の状況などによる使い分けが中心になると思われた．

KEY WORDS
・インフルエンザ
・(亜)型
・ノイラミニダーゼ阻害薬
・ウイルス残存
・IC_{50}

はじめに

2009年に初めて出現し同年秋を中心に大流行したA(H1N1) pdm09は，2010～2011年シーズンには冬期に流行し季節性化したが，同シーズンはA(H3N2)やB型も流行する混合流行であった．また抗インフルエンザ薬のノイラミニダーゼ(NA)阻害薬は，従来の内服薬のオセルタミビルと吸入薬のザナミビルに加えて，2009～2010年シーズン後半から点滴注射薬のペラミビル[1]，2010～2011年シーズンからは1回吸入で治療が完結するラニナミビル[2)3)]が使用可能となり，NA阻害薬4剤の時代を迎えた．なお，かつてよく使われたM2蛋白阻害薬のアマンタジンは現在，A(H3N2)およびA(H1N1) pdm09には耐性型とされ，ほとんど使用されていない．この4種類のNA阻害薬は投与経路や投与回数が異なるため，臨床医の選択肢が広がった一方で選択に迷う場面も考えられる．われわれは2010～2011年シーズンに初めてこの4種類のNA阻害薬の比較研究を行ったのでその結果をもとに，これらの使い方について考えてみたい．

1　4種類のNA阻害薬の使用状況

2010～2011年シーズンに日本臨床内科医会(日臨内)研究グループにおいて，迅速診断でA型かB型と判定された792例について各年齢層のNA阻害薬4剤の使用状況を検討したところ，次のような結果が得られた．

4歳以下では90例中，オセルタミビルが85例(94%)と圧倒的に高頻度に使用され，ほかはザナミビル3例，ラニナミビル2例が使用された．5～9歳では216例中，やはりオセルタミビルが122例(56%)と最も多く使用されたが，ラニナミビル(45例，21%)，ザナミビル(42例，19%)も比較的多く使われた．これに対してペラミビルは7例で使用されたにとどまった．

次いで10～19歳では211例中，ザナミビルが124例(59%)と最も多く，次いでラニナミビルが73例(35%)使用されてこの2剤が94%とほとんどを占めた．これらに比してオセルタミビル(8例)とペラミビル(6例)の使用例は少数にとどまった．20～59歳238例の使用薬剤としては，ラニナミビルが108例(45%)と最も多く，次いでオ

セルタミビル(81例，34%)，ザナミビル(26例，11%)，ペラミビル(23例，10%)の順であった．以上から年齢別には，2010～2011年シーズン，9歳以下はオセルタミビル，10～19歳はザナミビル，20～59歳はラニナミビルという使用傾向が示された．

また各薬剤について使用年齢構成をみると，図1のようになった．すなわちオセルタミビルの使用例は5～9歳が最も多く(39%)，次いで4歳以下(27%)，20～59歳(26%)の順で，10代と60歳以上は少なかった．ザナミビルの使用年齢は10～19歳が最も多く(61%)，次いで5～9歳，20～59歳の順であった．またペラミビルは20～59歳が最も多く(61%)，次いで5～9歳，10～19歳の順であった．さらにラニナミビルは20～59歳が最も多く(45%)，以下10～19歳(30%)，5～9歳(19%)の順であった．

この結果，2010～2011年シーズン，オセルタミビルは9歳以下，ザナミビルは10代，ペラミビルは20歳以上がそれぞれ6割以上を占めたが，ラニナミビルの使用例は成人がもっとも多いものの，5歳以上のいずれの年代でも比較的広く使用されていた．

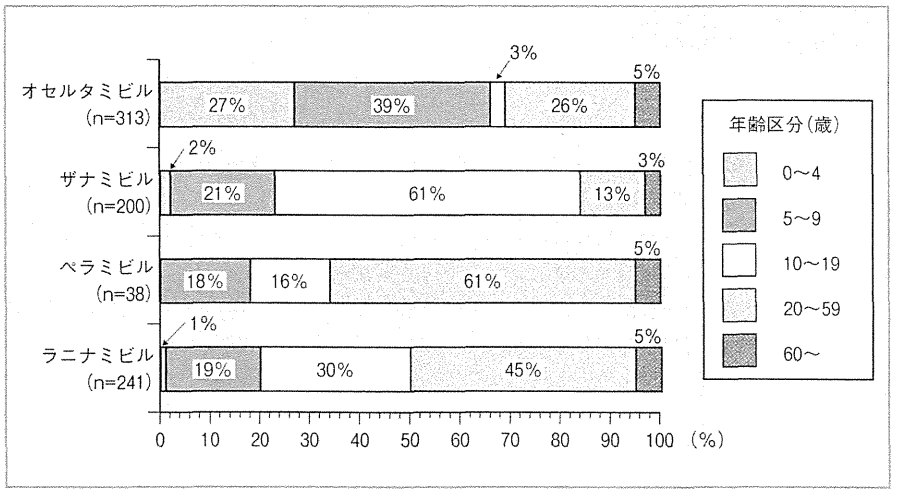
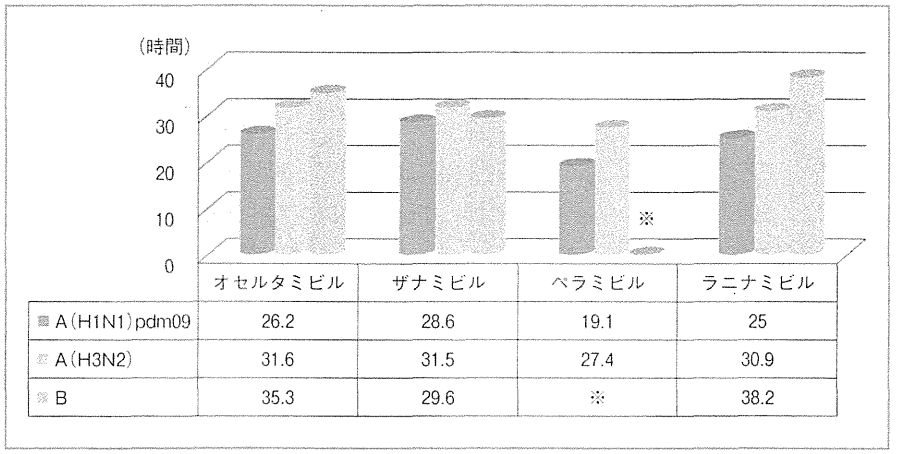


図1　各薬剤の使用年齢分布



	オセルタミビル	ザナミビル	ペラミビル	ラニナミビル
A(H1N1)pdm09	26.2	28.6	19.1	25
A(H3N2)	31.6	31.5	27.4	30.9
B	35.3	29.6	※	38.2

図2　各抗インフルエンザ薬における(亜)型別の平均解熱時間
※：少数例のため解析不能．

2　各NA阻害薬の亜型別の有効性

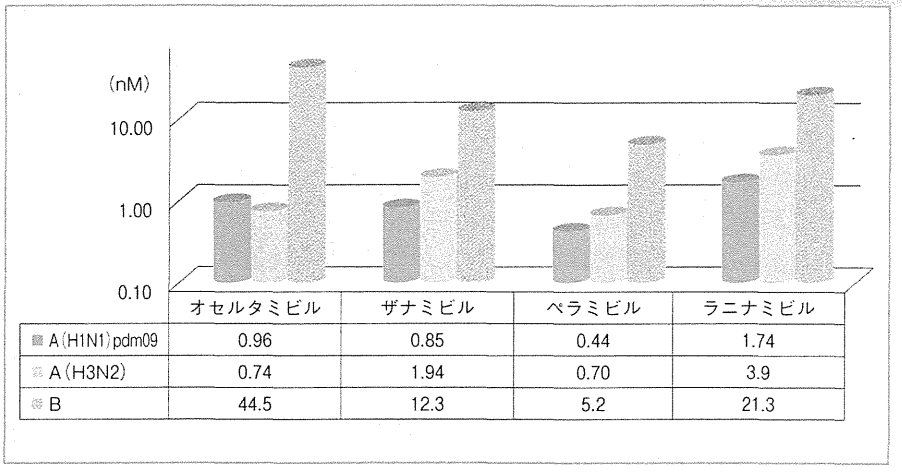
各NA阻害薬について，A(H1N1)pdm09，A(H3N2)，Bの各(亜)型別に平均の解熱時間(薬剤投与開始後37.5℃を切るまでの時間)を示したのが図2である．オセルタミビルとラニナミビルの解熱時間はいずれもA(H1N1)pdm09が最も短くおのおの26.2時間，25時間であり，次がA(H3N2)で，Bは最も解熱時間が長い傾向にあった．ペラミビルはB型が少数で解析不能であったものの，A(H1N1)pdm09がA(H3N2)よりも解熱時間が短い傾向にあるのはオセルタミビル，ラニナミビルと同様であった．またザナミビルの解熱時間は3(亜)型とも28.6～31.5時間で近似していたが，やはりA(H1N1)pdm09で最も短い傾向にあった．

3　各NA阻害薬に対するIC_{50}

この4剤のいずれかを投与され解熱時間の検討対象となった症例のうち，

治療開始前にウイルスが分離培養されIC$_{50}$（NA活性の50%阻害濃度）を測定できた61例について，各薬剤に対するIC$_{50}$を図3に示した．ザナミビル，ペラミビル，ラニナミビルはいずれもA（H1N1）pdm09が最も低く，A（H3N2），Bの順に高かった[4]．またオセルタミビルに対するIC$_{50}$はA（H3N2）が最も低かったがA（H1N1）pdm09との差はわずかで，これらに比してBでは著しく高かった．このようにIC$_{50}$が各薬剤ともにA（H1N1）pdm09で最も低い傾向にあって，かつBで最も高いという結果は，図2で各（亜）型に対するNA阻害薬の解熱時間がA（H1N1）pdm09で最も短く，一部の例外はあるもののBで長い傾向にあることとほぼ一致するものと思われた．

なおIC$_{50}$を各薬剤間で比較することはそれぞれのNA阻害薬の投与量，組織移行性，組織内分布などの差もあり，容易ではないと考えられる．事実，A（H1N1）pdm09において，ラニナミビルは解熱時間がオセルタミビル，ザナミビルなどよりも短い傾向にあったが，IC$_{50}$はこれら2剤の2倍前後と高かった．ラニナミビルは気道，肺の上皮細胞内（主にゴルジ体内）に長時間貯留して効果を発揮すると考えられ，細胞表面でNA阻害効果を発揮するほかのNA阻害薬とは異なった体内動態，作用形式を示すことから，IC$_{50}$で薬剤間の効果比較をすることは必ずしも妥当ではないことが示唆された．



	オセルタミビル	ザナミビル	ペラミビル	ラニナミビル
A（H1N1）pdm09	0.96	0.85	0.44	1.74
A（H3N2）	0.74	1.94	0.70	3.9
B	44.5	12.3	5.2	21.3

図3　PCRの各（亜）型におけるIC$_{50}$（平均値）
2010～2011年シーズン（抗インフルエンザ薬検討の61例について）．

表1　解熱時間，発熱時間に及ぼす影響因子（重回帰分析結果）

パラメーター	t値	p値
解熱時間		
（亜）型（PCR結果）	3.63	0.00033
最高体温	3.49	0.00055
発熱時間		
発症～投与開始（時間）	11.2	2.1×10^{-25}
最高体温	3.60	0.00036
（亜）型（PCR結果）	3.49	0.00054

いずれもワクチン接種回数，年齢，性別，前年のインフルエンザ罹患，NA阻害薬の種類はNS．また解熱時間については発症～投与開始間隔（時間）はNS．

4　4種類のNA阻害薬の効果に及ぼす影響因子

この4剤の解熱時間および発熱時間（発症から解熱までの時間）に及ぼす影響の有無を検討するために，NA阻害薬の種類，最高体温，PCRによる（亜）型，ワクチン接種回数，発症～投与開始までの経過時間，年齢，性別，前年のインフルエンザ罹患の各項目と解熱時間または発熱時間との重相関解析を行った．

表1に示すように，解熱時間は（亜）型および最高体温とは有意な相関を示したが（ともにp<0.001），これら以外の項目とは有意な相関を示さなかった．また発熱時間は発症～投与開始までの経過時間，（亜）型および最高体温とは有意な相関を示したが（ともにp<0.001），これら以外の項目とは有意

な相関を示さなかった．

以上の結果は，オセルタミビル耐性のみられなかったシーズンに行われたわれわれの過去の結果とほぼ一致している[5]．今回の解析でも，使用したNA阻害薬の種類は基本的には解熱時間や発熱時間に大きな影響はなく，むしろ(亜)型の種類のほうが影響することが示唆された．

5 ラニナミビル投与後のウイルス残存率

われわれはすでにオセルタミビル[6][7]，ザナミビル[8]については，各(亜)型における投与後5±1日目のウイルス残存率をウイルス培養法により検討し報告している．今回は2010～2011年シーズンにおけるラニナミビル投与後のウイルス残存率を過去のオセルタミビル，ザナミビルによる季節性インフルエンザのウイルス残存率と比較して表2に示した．ラニナミビルのウイルス残存率は，A(H1N1)pdm09は2.3%，A(H3N2)は10.5%，Bは29.4%であり，(亜)型別にみるとやはりA(H1N1)pdm09で最も低く，次いでA(H3N2)，Bの順であった．一方，過去のわれわれの成績でオセルタミビルはA(H1N1)ソ連型が11.4%，A(H3N2)が13.2%，Bが33.3%，ザナミビルはA(H1N1)ソ連型が20.0%，A(H3N2)が11.8%，Bが25.5%であり，今回得られたラニナミビルのウイルス残存率の成績は過去のオセルタミビルやザナミビルのウイルス残存率の結果と比べても遜色ないと考えられた[6]-[8]．

表2　ウイルス培養法によるラニナミビル投与後の(亜)型別ウイルス残存率(過去のオセルタミビル，ザナミビルとの比較，投与5±1日目)

	A(H1N1)	A(H3N2)	B
ラニナミビル	2.3%(2/86) (pdm09)	10.5%(2/19)	29.4%(5/17)
オセルタミビル	11.4%(5/44)※1 (ソ連)	13.2%(20/151)※2	33.3%(22/66)※2
ザナミビル※3	20.0%(2/10) (ソ連)	11.8%(12/102)	25.5%(26/101)

※1：2007～2008年シーズン．※2：2003～2004 & 2004～2005年シーズン．※3：2006～2007年シーズン．

6 NA阻害薬の使い方

最後に各NA阻害薬の使用法を表3に示した．前述のように(亜)型別には若干有効性が異なる可能性があるものの，4剤のNA阻害薬間で全体としては大きな効果の違いはあまりないと考えられる．しかし，この4剤は投与経路(経口，吸入，静脈注射)，投与回数(1回，あるいは1日2回5日間)，耐性の状況などを考慮した使い分けが必要と思われる．また吸入薬(特に1回完結型のラニナミビル)は幼小児ではきちんと吸入されないと本来の効果を発揮できない可能性があり，吸入指導がきわめて重要となる．以下，各NA阻害薬の使い分けのポイントを記す．

1. オセルタミビル

1日2回5日間投与の内服薬で，10歳以上の未成年では因果関係は定かではないものの異常行動の懸念によって，ハイリスク者を除いて原則使用禁止措置が続いている．2008～2009年シーズンにA(H1N1)ソ連型ウイルスが100%，H275Y変異によって本薬に耐性となり，特に小児で有効性が低下した[7][9]．現在流行中のA(H1N1)pdm09もH275Y変異が起きると本薬の有効性低下が懸念されるが，幸い現状での耐性化率は1～2%と低く本薬の有効性は高い[4]．今後も広範囲に耐性化が起きない限り，特に吸入困難な4歳以下の小児を中心に頻用されると思われる．

2. ザナミビル

1日2回5日間投与の吸入薬であり，高濃度でウイルス増殖部位に直接作用する．耐性ウイルスの報告は少なく，H275Y変異のA(H1N1)型例にも有効で，かつB型を含めたいずれの(亜)型にも有効性が高い[8][9]．本薬剤はプロドラッグでなく，活性物質そのものであり，速効性に優れる可能性がある[10]．今後も10代を中心に広く使用されると思われる．

3. ペラミビル

米国より導入された点滴注射薬．成人300 mg，小児10 mg/kgを15分以上かけて単回投与するが，重症化の恐

表3　ノイラミニダーゼ阻害薬の使用法

	オセルタミビル	ザナミビル	ペラミビル	ラニナミビル
商品名	タミフル	リレンザ	ラピアクタ	イナビル
剤形	内服薬	吸入薬	点滴注射薬	吸入薬
用法・用量	Cap：1回1Cap(75 mg) DS：1回2 mg/kg(1回最高75 mg) 1日2回5日間内服	1回10 mg (5 mgブリスターを2個) 1日2回5日間吸入	成人：300 mg 小児：10 mg/kg 単回点滴(重症化の恐れがある場合は1日最大600 mgや連日の投与可)	10歳以上：40 mg (20 mgを2容器) 10歳未満：20 mg (20 mgを1容器) 単回吸入
予防投薬の適用	1CapまたはDS2 mg/kg(最高75 mg)を1日1回	10 mg 1日1回	なし	なし
H275Y変異の影響	(+)	(−)	(±)	(−)
副作用	胃腸障害など	まれ	下痢	まれ
特記事項	現状でH275Y変異ウイルスは少なく，原則使用不可の10代を除いて広い年代で有用．特に4歳以下(1歳未満を除く)の治療の中心．	耐性はほとんどみられずH275Y変異例，B型例を含め，いずれの(亜)型でも有効性が高く，外来の10代の患者を中心によく使われている．	すべての年齢で使えるが，特に入院重症例を含めて，内服や吸入ができないなどの患者では最適．	1回完結型のためコンプライアンスに優れ，服用忘れが多い成人などには特に適する．幼小児では吸入指導が重要．

れがある場合はともに1日1回600 mgまでの使用や連日の投与が可能[1]．H275Y変異のA(H1N1)型例で感受性低下が報告されている[11]．入院重症例を含め，経口や吸入が困難な症例では第一選択となる．

4．ラニナミビル

純国産で長時間作用型の吸入薬(プロドラッグ)．発症後1回の投与で気道や肺に長時間貯留し，5日間投与のオセルタミビルと同等の薬効を示す．H275Y変異型にも有効．1回完結型のため，外来患者の利便性は高く症状改善による服薬中止の心配もないが，1回投与であるがゆえに，特に幼小児では吸入指導がきわめて重要となる．コンプライアンスの悪い成人などを中心に今後さらに使用率が高まると思われる．

おわりに

NA阻害薬は4種類になって，患者の状況，年齢，本人の希望などによって幅広い選択が可能になってきた．しかし毎年のように流行ウイルスも変化しており，特にH275Y変異など耐性化の動向は目が離せない状況にある．今後も流行の状況に細心の注意を払い，その時々で的確な選択が望まれる．

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今シーズンにおける抗インフルエンザ薬の使い方

ウイルスの薬剤耐性化への懸念は不要

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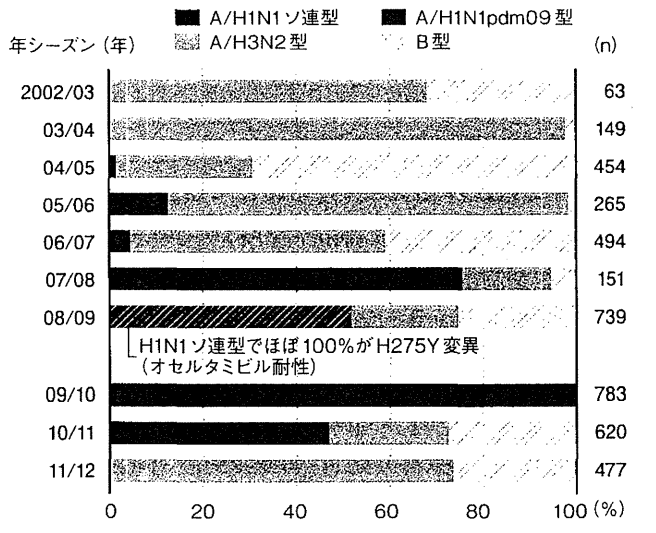
2009年に世界的に大流行したパンデミックウイルス、A/H1N1pdm09（A/pdm09型）は、10/11年シーズン後に季節性化した。その後も毎年流行が続くという予測もあったが、11/12年には国内でほとんど姿を見せず、A/H3N2型が流行の主流に戻った。このA/H3N2型はM2蛋白阻害薬アマンタジンに対して大半が耐性を獲得している。また現在、承認申請中のRNAポリメラーゼ阻害薬（ファビピラビル）も使用できない。従って、今シーズンのインフルエンザに対しても、ノイラミニダーゼ（NA）阻害薬が治療の中心となる。NA阻害薬は4剤が使用できるようになって3シーズン目を迎える。

図1　過去10シーズンにおけるインフルエンザの型（亜型）別内訳



（文献1から引用）

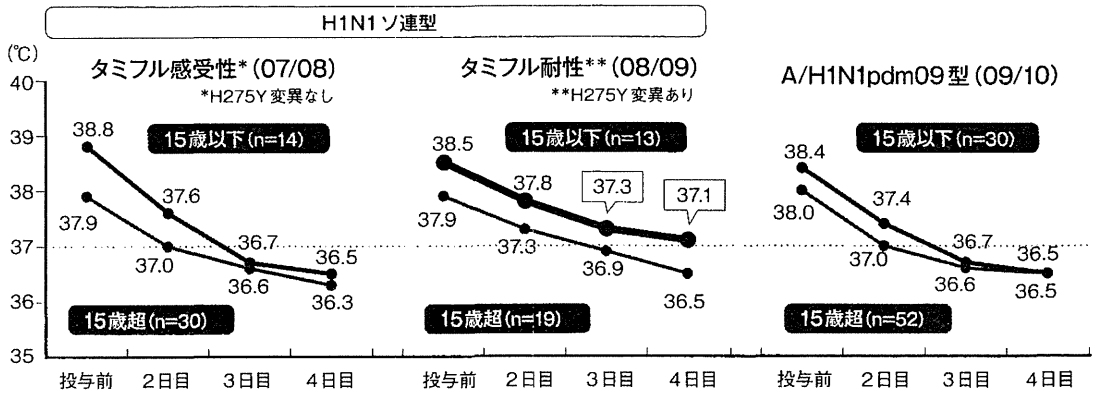
近年の流行状況とNA阻害薬耐性化の現状

図1に過去10シーズンにおけるウイルス型（亜型）を示す。特にA型は06/07年まではH3N2型主体だったのが、07/08年から10/11年はH1N1型主体になった。このH1N1型も各シーズンで様相を異にし、07/08年はオセルタミビル感受性のソ連型、08/09年はほぼ100％がH275Y（H274Yとも表記される）変異によるオセルタミビル耐性ソ連型、そして09/10年にはソ連型が全く消えて、H1N1亜型はほぼ全てpdm09型に置き換わった。その後pdm09型は11/12年に国内でほとんど見られなくなったが[1]、海外ではまだ流行している地域もある。

11年8月以降、pdm09型とは異なる新種のブタインフルエンザウイルス「variant H3N2（H3N2v）」が米国内で散発的に発生している。米疾病対策センター（CDC）によれば、H3N2vは現在はブタからの直接感染が主で、症状は季節性に似ている。これまで累計で300人以上（死亡1人）の患者が確認されている（11月現在）。本ウイルスはpdm09ウイルス遺伝子の一部を有しており、ヒトからヒトへの感染に移行した場合、強い感染力で流行が拡大する可能性が否定できず、今後の動向に注意が必要である。H3N2vに対してオセルタミビル、ザナミビルなどのNA阻害薬は有効とされている。

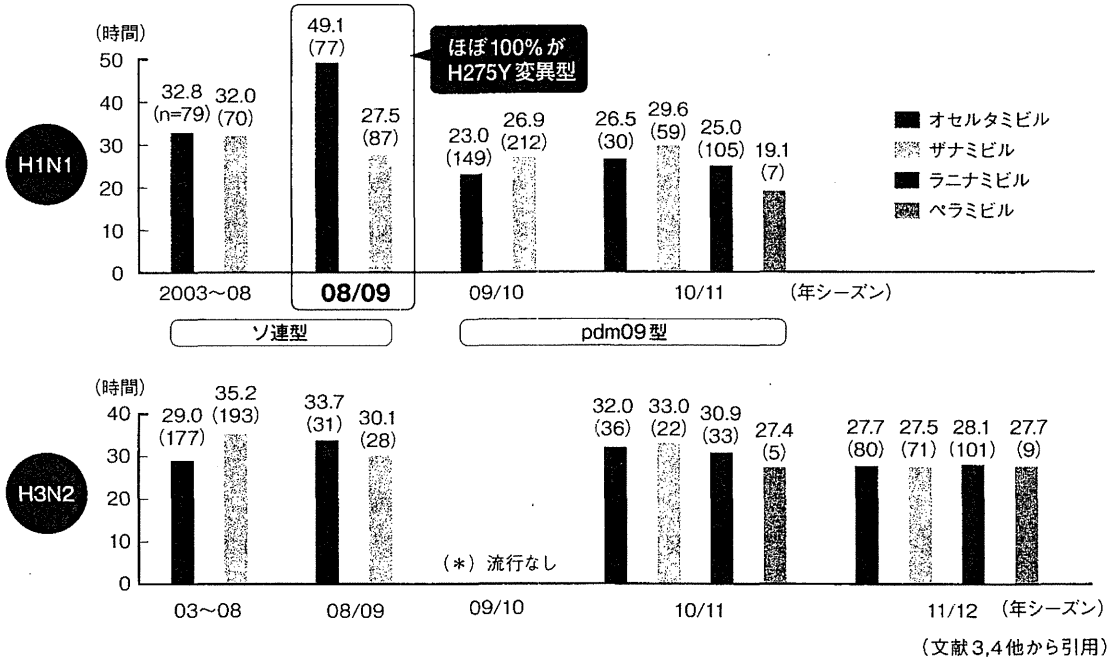
B型は、08/09年までほぼ隔年で流行したが、10/11年と11/12年は続けて流行し、11/12年はそれまでのビクトリア系統に、山形系統が加わってきた。

図2　08/09年と前後のシーズンにおけるオセルタミビル投与開始前後の平均体温の推移



（文献2から引用）

図3　A/H1N1型とA/H3N2型における平均解熱時間の経年推移



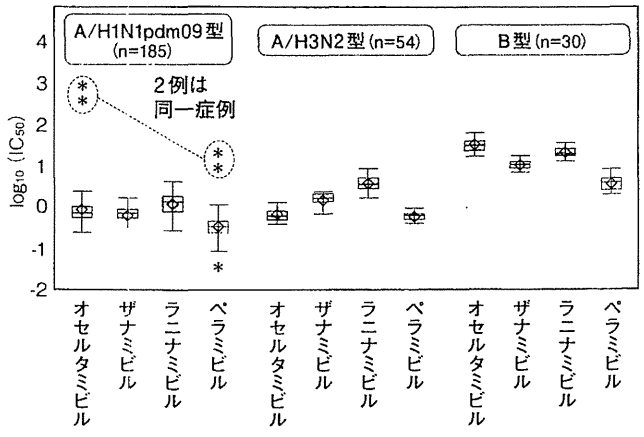
（文献3,4他から引用）

われわれは、08/09年のオセルタミビル耐性ソ連型では同薬投与後も、特に小児で発熱が遷延することを報告した。ただし、翌年のpdm09型の登場とともにこのソ連型は消失した。同じH1N1亜型でもpdm09型では同薬投与後の発熱遷延は見られず、有効性が戻った（図2）[2]。

A亜型における過去10シーズンのオセルタミビルとザナミビル、過去2シーズンのラニナミビル、ペラミビルの有効性の推移を解熱時間で図3に示す。08/09年のソ連型におけるオセルタミビルを除いて、いずれの薬剤も現在に至るまで有効性が高い[3,4]。

われわれの10/11年の調査では、pdm09型において185人中2人のみ培養ウイルスのオセルタミビルに対するIC50（NA活性の50％阻害濃度）が異常高値を示した[5]。この2例はH275Y変異を有することが確認されたが、耐性化率としては1.1％（185人中2人）にとどまった（図4）。この2例はペラミビルでも軽度のIC50の上昇を示した。しかしわれわれは、H3N2型やB型ではいずれのNA阻害薬でも、10/11年、11/12年の両シーズンでIC50が上昇していないことを確認している。現在、NA阻害薬への耐性化はほとんど問題ないと考えられる。

図4　10/11年の各型におけるNA阻害薬のIC50値



◇算術平均値　＊箱から3四分位範囲より離れた値

（文献5から引用）

NA阻害薬4剤の使い分けのポイント

11/12年のH3N2亜型では、NA阻害薬4剤の解熱時間はほぼ同じだった（図3）。また、同シーズンに培養で確定したB型例の解熱時間は、症例数が少ないペラミビルを除くとザナミビルがやや短い（平均31.6時間、n=28）が、オセルタミビル（37.8時間、n=25）とラニナミビル（38.5時間、n=24）ではほとんど差がなかった。

なお予防投与は、オセルタミビルとザナミビルに適応（ただし自費診療）があり、ラニナミビルは現在申請中である。

(1) オセルタミビル

1日2回5日間服用の内服薬。10歳以上の未成年では因果関係は定かではないものの、異常行動の懸念からハイリスク者を除いて原則使用禁止状態が続いている。本薬剤に耐性を示すウイルスは現在はほぼ消失しており、現状では有効性は他のNA阻害薬と同様に高い。本薬は1歳未満を除いて、吸入薬の使用が困難な3、4歳以下の幼児において実質的に第一選択薬となっている。

(2) ザナミビル

1日2回5日間使用の吸入薬。登場後10年以上にわたって耐性ウイルスの報告はほとんどなく、いずれのウイルス型（亜型）にも有効性が高い。4歳以下の安全性は確認されていない。ラニナミビルほどは1回の吸入成否による治療効果への影響が大きくなく、オセルタミビルが使いにくい10歳代を中心によく使用されている。

(3) ラニナミビル

日本で開発された長時間作用型の吸入薬（プロドラッグ）。ザナミビルと同様に耐性ウイルスはまれであり、1回完結型のため外来患者の利便性が高い。また、5日間投与の薬剤と異なり症状改善による服薬中止の心配もなく、多忙のため服薬コンプライアンスにやや欠ける成人でよく使用されている。ただし1回吸入であるが故に、特に幼小児では吸入指導が極めて重要で、自宅よりも院内や調剤薬局などで吸入させるのが望ましい。5歳以上であれば吸入可能と思われるが、吸入力に不安がある小児低年齢患者では、吸入確認用ツール（笛）も用意されている。

(4) ペラミビル

米国から導入された点滴注射薬で、成人300mg、小児10mg/kgを15分以上かけて単回投与する。重症化の恐れがある場合は、ともに1日1回600mgまでの投与や連日投与が可能である。特に経口や吸入が困難な症例、入院例、重症例などでは年齢を問わず使用が推奨される。

おわりに

NA阻害薬4剤時代になり抗インフルエンザ薬の選択肢が広がり、患者の病態に合わせた薬剤選択が可能になりつつある。だが、インフルエンザウイルスは毎年のように流行型が変化し、オセルタミビル耐性ソ連型やA/pdm09型の登場など、予想外のことがしばしば起きている。本稿に記載した治療選択肢も、今後の流行や耐性化の推移により変化する可能性があることを書き添えて稿を終えたい。

[参考文献]

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