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Original article

In vitro neuraminidase inhibitory activity of four neuraminidase inhibitors against clinical isolates of influenza virus in the Japanese 2012–2013 season

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ABSTRACT

The neuraminidase inhibitors (NAIs) oseltamivir phosphate (Tamiflu®), zanamivir (Relenza®), laninamivir octanoate (Inavir®), and peramivir (Rapiacta®) have been available for the treatment of influenza in Japan since 2010. The emergence of resistant virus to any of the NAIs is a great concern for influenza treatment. To assess the extent of viral resistance, we measured the 50% inhibitory concentration (IC_{50}) of each NAI for influenza virus isolates in the 2012–2013 influenza season and compared the results to those of the 2010–2011 and 2011–2012 influenza seasons.

Viral isolation of specimens obtained prior to treatment was done using Madine-Darby canine kidney cells, and the type and subtype of influenza, A(H1N1)pdm09, A(H3N2), or influenza B, was determined by RT-PCR using type- and subtype-specific primers. The IC_{50} was determined by a neuraminidase inhibition assay using a fluorescent substrate.

A total of 329 influenza viruses were isolated:5 influenza A(H1N1)pdm09 (1.5%), 316 influenza A(H3N2) (96.1%), and 8 influenza B (2.4%). No isolate showed an IC_{50} value exceeding 50 nM for any of the neuraminidase inhibitors. The IC_{50} values for A(H3N2) and B were similar to those of the 2010–2011 and 2011–2012 seasons. No isolate showed an increased IC_{50} value for A(H1N1)pdm09. These results indicate that the currently epidemic influenza viruses are susceptible to all four neuraminidase inhibitors, with no trend for IC_{50} values to increase at present.

1. Introduction

Treatment of influenza with the neuraminidase inhibitors (NAIs) oseltamivir phosphate (Tamiflu®), zanamivir (Relenza®), laninamivir octanoate (Inavir®), and peramivir (Rapiacta®) has become common among Japanese primary care providers [1–6].

The epidemic types/subtypes of influenza change every year. In the 2008–2009 season, almost 100% of the influenza A(H1N1) viruses were H275Y mutants with oseltamivir resistance. The oseltamivir-resistant A(H1N1) disappeared in the 2009–2010 season, replaced by an A(H1N1)pdm09 strain that was susceptible to oseltamivir. In the 2011–2012 season, A(H1N1)pdm09 was replaced by A(H3N2) and influenza B [7]. In order to select the optimal NAI for clinical use, it is important to monitor the susceptibility of the different influenza virus strains to each NAI.

We previously reported the IC_{50} of NAIs during the Japanese 2010–2011 and 2011–2012 influenza seasons [8,9]. In this study, we investigate the susceptibility of influenza virus to four NAIs in the 2012–2013 season and compare the results with those of the 2010–2011 and 2011–2012 seasons.

2. Materials and methods

2.1. Viral specimens

A total of 30 clinics and one hospital from 14 prefectures (Fukushima, Tokyo, Kanagawa, Chiba, Saitama, Gunma, Gifu, Kyoto, Ishikawa, Hyogo, Kagawa, Tokushima, Fukuoka and Kumamoto) participated in this study. Patients were enrolled from November 1, 2012 to April 30, 2013. Specimens for viral isolation were collected with informed consent from patients who showed a positive result with a rapid influenza antigen detection kit.

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2.2. Influenza virus isolation

Nasal aspirates, nasopharyngeal swabs, or self-blown nasal discharge were obtained for influenza virus isolation. The isolation was done as reported previously [8].

2.3. Viral typing/subtyping

The type and subtype of influenza A(H1N1)pdm09, A(H3N2), or influenza B was determined by RT-PCR using RNA extracted from the clinical specimens and type- and subtype-specific primers [10].

2.4. Measurement of susceptibility of NAIs to virus isolates

The 50% inhibitory concentration (IC_{50}) of oseltamivir carboxylate, zanamivir, laninamivir, and peramivir for each influenza isolate was determined by a fluorescence-based neuraminidase inhibition assay. The viruses were added to give 10–100 pmol/min of the neuraminidase reaction product for each assay, and IC_{50} was measured as described elsewhere [8]. We used the A/Yamagata/32/89 strain as the assay control to verify the assay results. Laninamivir and zanamivir were provided by Daiichi Sankyo Co., Ltd. (Tokyo, Japan). Oseltamivir carboxylate (oseltamivir) with 98.9% of purity was prepared from alkaline hydrolyzed oseltamivir phosphate (Tamiflu®, Chugai Pharmaceutical Co., Ltd., Tokyo, Japan) according to previously reported procedures [11]. Peramivir was obtained from a commercially available product (Rapiacta®, Shionogi & Co., Ltd., Osaka, Japan).

2.5. Statistical analysis

Differences in the age distribution of the A(H1N1)pdm09, A(H3N2), and B patient groups were tested by analysis of variance (ANOVA). A P value < 0.05 was considered statistically significant. Quantitative data were tabulated to provide descriptive summary statistics. The geometric means and 95% confidence intervals (CI) of IC_{50} for the four NAIs were calculated, and log-transformed IC_{50} values were compared between the two previous seasons and the 2012–2013 influenza season by the t-test. All analyses were performed using SAS system Release 9.2 software (SAS Institute Inc., Carey, NC, USA).

3. Results

A total of 329 influenza viruses were isolated in the 2012–2013 influenza season, including 5 influenza A(H1N1)pdm09 (1.5%), 316 influenza A(H3N2) (96.1%), and 8 influenza B (2.4%). The age distribution and viral type/subtype of the patients are shown in Table 1. The mean age was 26.0 ± 19.6 years, with no significant difference between males and females. The mean ages of patients with A(H1N1)pdm09, A(H3N2), and B were 35.8(±3.5), 25.9(±19.9), and 26.9(±15.5) years, respectively, with no significant difference among the three groups.

The geometric means of IC_{50} values for A(H1N1)pdm09, A(H3N2), and B to the four NAIs in the 2010–2011, 2011–2012, and 2012–2013 influenza seasons are listed in Table 2. No virus isolates in the 2012–2013 season showed high a IC_{50} value, over 50 nM, to any of the NAIs. All of the IC_{50} geometric mean values of the four NAIs for influenza B virus were much higher than those for A(H1N1)pdm09 and A(H3N2).

The ratios of the geometric mean values of IC_{50} for A(H1N1)pdm09 in the 2012–2013 to those in the 2010–2011 season were 0.98, 1.87, 1.57 and 2.01 for oseltamivir carboxylate, zanamivir, laninamivir, and peramivir, respectively. The increase in the IC_{50} values for zanamivir and peramivir were significant ($P = 0.0007$ and $P = 0.0216$, respectively). The ratios of the geometric mean values of IC_{50} for A(H3N2) in the 2012–2013 to those in the 2010–2011 season were 1.46, 1.49, 1.45, and 1.47 for the four NAIs, respectively. The ratios in the 2012–2013 to those in the 2011–2012 season were 1.38, 1.24, 1.35, and 1.17. Although the increase in the IC_{50} values of the four NAIs were slight, all were significant ($P < 0.0001$). In the case of influenza B virus, the ratios of the geometric mean values of IC_{50} in the 2012–2013 to those in the 2011–2012 season were 1.25, 2.02, 1.34, and 1.35, respectively. These increases in the IC_{50} values for zanamivir, laninamivir, and peramivir were significant ($P < 0.0001$, $P = 0.0298$, and $P = 0.0012$, respectively). The ratios of the geometric mean values of IC_{50} in the 2012–2013 season to those in the 2010–2011 season were 0.58, 1.31, 1.01, and 0.98, respectively. IC_{50} values were significantly decreased for oseltamivir and significantly increased for zanamivir. No significant differences were found for laninamivir and peramivir in the 2012–2013 and 2010–2011 seasons.

4. Discussion

We isolated 316 influenza A(H3N2) (96.1%), 8 influenza B (2.4%), and 5 influenza A(H1N1)pdm09(1.5%) viruses in the Japanese 2012–2013 influenza season. The percentage of virus types and subtypes was quite comparable to those reported by the Japanese National Institute of Infectious Disease [7].

The geometric means of the IC_{50} values for B to the four NAIs were consistently much higher than those for A(H1N1)pdm09 and A(H3N2) in the 2010–2011, 2011–2012, and 2012–2013 influenza seasons. Structure and activity studies of the carboxamide

Table 1
Age distribution of patients in the 2012–2013 seasons, by virus type.

Age group	No. of patients	Males	Females	A(H1N1)pdm09	A(H3N2)	B
0–9	75	47	28	0	74	1
10–19	90	40	50	0	88	2
20–29	41	24	17	0	40	1
30–39	38	21	17	4	32	2
40–49	37	16	21	1	34	2
50–59	22	8	14	0	22	0
60–69	16	5	11	0	16	0
70–79	6	4	2	0	6	0
80–	4	2	2	0	4	0
Total	329	167	162	5	316	8
Mean age ± SD (yrs)	26.0 ± 19.6	24.3 ± 18.9	27.8 ± 20.2	35.8 ± 3.5	25.9 ± 19.9	26.9 ± 15.5
		$P = 1.1051$		$P = 0.5294$		