J Infect Chemother xxx (2018) 1–6

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original Article

In vitro neuraminidase inhibitory concentration (IC_{50}) of four neuraminidase inhibitors in the Japanese 2016–17 season: Comparison with the 2010–11 to 2015–16 seasons☆

Hideyuki Ikematsu [a, *], Naoki Kawai [a], Norio Iwaki [a], Seizaburo Kashiwagi [a], Yusuke Ishikawa [b], Hiroki Yamaguchi [b], Kazuhito Shiosakai [b]

[a] Japan Physicians Association, Tokyo, Japan
[b] Daiichi Sankyo Co., Ltd, Tokyo, Japan

ARTICLE INFO

Article history:
Received 6 March 2018
Received in revised form
9 April 2018
Accepted 12 April 2018
Available online xxx

Keywords:
Influenza virus
Neuraminidase inhibitor
50% inhibitory concentration
Resistance
Surveillance

ABSTRACT

To assess the extent of susceptibility to the four most commonly used neuraminidase inhibitors (NAIs) in the viruses epidemic in the 2016–17 Japanese influenza season, we measured the 50% inhibitory concentration (IC_{50}) of these NAIs for influenza virus isolates from patients and compared them with the results from the 2010–11 to 2015–16 seasons.

Viral isolation was done with specimens obtained prior to treatment, and the type and subtype 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 276 virus isolates, 6 A (H1N1)pdm09 (2.2%), 249 A (H3N2) (90.2%), and 21 B (7.6%), had the IC_{50} measured for the four NAIs. B isolates included 11 (52.4%), 9 (42.9%), and one (4.8%) of the Victoria, Yamagata, and undetermined strains, respectively.

No A (H1N1)pdm09 with highly reduced sensitivity for oseltamivir was found in the 2016–17 season. No isolate with highly reduced sensitivity to the four NAIs have been found for A (H3N2) or B from the 2010–11 to 2016–17 seasons. No significant trend of increase or decrease was found in the geometric mean IC_{50}s of the four NAIs during the seven studied seasons.

These results indicate that the sensitivity to the four commonly used NAIs has been maintained and that any change in the effectiveness of these NAIs would be minute. Common usage of NAIs for patient treatment has not been a driving force in the selection of NAI resistant viruses.

1. Introduction

Treatment of influenza with the neuraminidase inhibitors (NAIs) oseltamivir phosphate (Tamiflu®, oseltamivir), zanamivir hydrate (Relenza®, zanamivir), peramivir hydrate (Rapiacta®, peramivir), and laninamivir octanoate hydrate (Inavir®, laninamivir) has become common among Japanese primary care providers [1–3]. Thus, the possible emergence of resistant virus to any of the NAIs is of great concern for physicians. A prolonged duration of fever after the administration of oseltamivir was confirmed in patients who had been infected with oseltamivir resistant A (H1N1) viruses in the Japanese 2008-09 season [4,5]. The presence of oseltamivir resistant A (H1N1)pdm09 from the 2009–10 season has been reported by National Institute of Infectious Diseases in Japan [1]. Our long-term survey of virus sensitivity to NAIs from the 2010-11 season found oseltamivir resistant A (H1N1)pdm09 virus in 2 of 185 (1.1%), 2 of 172 (1.2%), and 2 of 210 (0.95%) patients in the 2010–11, 2013–2014 and 2015–16 seasons, respectively [6–11]. The frequency of oseltamivir resistant A (H1N1) increased to almost 100% in the 2008-09 season, but A (H1N1)pdm09 did not.

Global surveillance of the susceptibility of human influenza virus to NAIs is important [12,13]. However, the background of the medical conditions of other countries, such as antiviral usage, may be different from that of the clinical setting in Japan where NAIs are

☆ All authors meet the ICMJE authorship criteria.

* Corresponding author. 2-18-30 Hakataekihigashi, hakata-ku, Fukuoka, 812-0013, Japan.
E-mail address: ikematsu@gray.plala.or.jp (H. Ikematsu).

https://doi.org/10.1016/j.jiac.2018.04.009
1341-321X/

commonly used. In this study, we investigate the IC_{50}s of the 2016–2017 season using virus isolated from influenza patients who visited the clinic participating this study and compare the results with those we have reported for the previous six influenza seasons, from 2010 to 11 to 2015–16.

2. Patients and methods

2.1. Patients

This study was done as part of a post-marketing surveillance of laninamivir according the rules of the Ministry of Health and Labor of Japan. A total of 28 clinics and 1 hospital from 13 prefectures participated (Fukushima, Tokyo, Chiba, Saitama, Kanagawa, Gunma, Gifu, Ishikawa, Hyogo, Kagawa, Tokushima, Fukuoka, Kumamoto), which was almost identical to the previous seasons. Patients were enrolled from November 2016 to April 2017. Specimen for viral isolation were collected with informed consent from patients who showed a positive result with a rapid influenza antigen detection kit. Informed consent was obtained from a parent if the patient was under 19 years.

2.2. Influenza virus isolation and typing

Nasal aspirates, nasopharyngeal swabs, or self-blown nasal discharge were obtained from patients prior to treatment. Influenza virus isolation was done from these specimen with Madin-Darby canine kidney (MDCK) cells. The type and subtype of influenza was determined by RT-PCR using RNA extracted from the clinical specimen. Type- and subtype-specific primers were used [14]. The Yamagata and Victoria lineages of influenza B virus were discriminated by real-time RT-PCR using specific primers and probes for the hemagglutinin gene [15].

2.3. Measurement of susceptibility of NAIs to virus isolates

As a marker of virus susceptibility to NAIs, the 50% inhibitory concentration (IC_{50}) of oseltamivir, zanamivir, peramivir, and laninamivir were determined for each influenza isolate by a fluorescence-based neuraminidase inhibition assay [16]. The influenza virus strain A/Yamagata/32/89(H1N1) was included as the reference strain in each assay. The IC_{50} results of each season from 2010 to 2011 to 2015–2016 were compared. Our definition of highly reduced sensitivity follows the statement by the World Health Organization (WHO) that influenza virus with an IC_{50} increase of 100 fold for type A or 50 fold for type B compared with the IC_{50} of the reference viruses indicates highly reduced sensitivity [17].

2.4. Statistical analysis

Differences in the age distribution of the B patients with the A (H1N1)pdm09 and A (H3N2) were determined by *t*-test. The geometric mean (GM) and 95% confidence interval (CI) of the IC_{50} of each of for the four NAIs were calculated, and log-transformed IC_{50} values were compared among the viral type/subtypes or the seven seasons by ANOVA. The difference between the IC_{50}s of the two B lineages was culculated by *t*-test. A *P* value <0.05 was considered statistically significant. All analyses were performed using SAS system Release 9.2 software.

3. Results

3.1. Samples

In the 2016–2017 season, 340 specimens were collected and cultured with MDCK cells. Insufficient virus growth in MDCK cells was observed for 60 specimens, and their IC_{50}s were not able to be measured. The virus type/subtype was undetermined for four isolates, leaving a total of 276 available for analysis; 6 influenza A (H1N1)pdm09 (2.2%), 249 A (H3N2) (90.2%), and 21 B (7.6%). The B group included 11 Victoria (52.4%), 9 Yamagata (42.9%), and one undetermined.

The patient age distribution by viral type/subtype is shown in Table 1. The overall mean age was 27.7 ± 21.4 years. The mean age of the patients with B (18.1 ± 15.5 years) was lower than that of A (H1N1)pdm09 and A (H3N2) (36.2 ± 28.7 and 28.3 ± 21.5 years, respectively), with the difference among viral type/subtypes statistically significant ($p = 0.0498$ and $p = 0.0361$, *t*-test).

3.2. Virus with highly reduced sensitivity to NAIs

Distribution of the IC_{50} values of the four NAIs in the 2016-17 season are depicted by virus type/subtype using Box and Whisker plot analysis along with the results of the seasons from 2010 to 11 to 2015–16: for A (H1N1)pdm09 in Fig. 1A, for A (H3N2) in Fig. 1B, and for B in Fig. 1C.

No A (H1N1)pdm09 virus with highly reduced sensitivity was observed in the 2016-17 season, although virus with highly reduced sensitivity for oseltamivir was found in the (2010–11, 2013–14, 2015–16 seasons).

Table 1
Patient age distribution in the 2016/17 season by virus type/subtype.

Age group	Virus type/subtype			Total
	A(H1N1)pdm09 No. of patients	A(H3N2) No. of patients	B No. of patients	
0–9	2	37	9	48
10–19	1	92	6	99
20–29	0	28	1	29
30–39	0	21	4	25
40–49	0	25	0	25
50–59	2	18	0	20
60–69	0	15	1	16
70–79	1	6	0	7
80-	0	7	0	7
Total	6	249	21	276
Mean age ± SD, yrs	36.2 ± 28.7	28.3 ± 21.5	18.1 ± 15.5	27.7 ± 21.4

Please cite this article in press as: Ikematsu H, et al., In vitro neuraminidase inhibitory concentration (IC_{50}) of four neuraminidase inhibitors in the Japanese 2016–17 season: Comparison with the 2010–11 to 2015–16 seasons, J Infect Chemother (2018), https://doi.org/10.1016/j.jiac.2018.04.009