Short Communication

Oseltamivir-Resistant Seasonal A(H1N1) and A(H1N1)pdm09 Influenza Viruses from the 2007/2008 to 2012/2013 Season in Nara Prefecture, Japan

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SUMMARY: We examined the incidence of oseltamivir-resistant seasonal A(H1N1) and A(H1N1)pdm09 influenza viruses from the 2007/2008 to 2012/2013 season in Nara Prefecture, Japan. To detect the oseltamivir resistance marker in neuraminidase (NA), 365 influenza viruses (60 seasonal A(H1N1) and 305 A(H1N1)pdm09) were sequenced. The H275Y mutation in the NA gene, which confers resistance to oseltamivir, was identified in 93.8% (30/32) of seasonal A(H1N1) viruses that were circulating during the 2008/2009 season. Moreover, the detection rate of oseltamivir-resistant A(H1N1)pdm09 viruses was 4.1% (3/74) and 2.8% (5/180) in the 2009/2010 and 2010/2011 season, respectively. Four cases of oseltamivir-resistant A(H1N1)pdm09 virus infection occurred in the same hematology ward during the 2010/2011 season. Our data show a low frequency of oseltamivir-resistant A(H1N1)pdm09 virus in Nara Prefecture but suggested the possibility of human-to-human transmission of this virus.

Oseltamivir has been widely used for many years as a treatment for influenza virus infections. In Japan, oseltamivir was approved for the treatment of influenza patients in 2000. Japan has the highest annual usage of oseltamivir in the world. Until the 2006/2007 influenza season, oseltamivir-resistant influenza viruses were rarely observed worldwide (1). However, during the 2007/2008 influenza season, the prevalence of oseltamivir-resistant seasonal A(H1N1) viruses with the H275Y mutation was reported, mainly in European countries (2). In Japan, oseltamivir-resistant seasonal A(H1N1) viruses were widespread during the 2008/2009 season (3,4). Although A(H1N1)pdm09 viruses that emerged in 2009 have been reported to be sensitive to oseltamivir, oseltamivir-resistant A(H1N1)pdm09 viruses have been sporadically detected (5–7).

In Japan, few reports have described the continuous monitoring of oseltamivir-resistant influenza viruses at the community level (8,9). The accumulation of such data at the community level is important to elucidate the distribution of oseltamivir-resistant influenza viruses in Japan. This study presents the characterization of oseltamivir-resistant seasonal A(H1N1) and A(H1N1)pdm09 influenza viruses from the 2007/2008 to 2012/2013 season in Nara Prefecture, Japan.

For the present study, clinical specimens were collected from Nara Prefecture. A total of 994 influenza viruses (92 seasonal A(H1N1) and 902 A(H1N1)pdm09) were detected mainly using real-time reverse transcription-PCR assay (10) from the 2007/2008 to 2012/2013 season. Oseltamivir-resistant influenza viruses was detected by reverse transcription-PCR and DNA sequenc-

ing of the neuraminidase (NA) gene as previously described (3,5,11). The supernatants of throat swabs were inoculated into MDCK cells prepared in 48-well multiplates for influenza virus isolation. After virus isolation, NA inhibition assay and sequence analysis were performed for representative isolates and specimens at the National Institute of Infectious Diseases, Tokyo, Japan. Further, an NA inhibition assay was performed to ascertain the susceptibility of viruses to NA inhibitors, as described in a previous report (7). The nucleotide sequences of the full-length NA gene and HA1 domain of the hemagglutinin (HA) gene were subjected to phylogenetic trees constructed using software (MEGA 5.0) (12). The phylogenetic trees were obtained using the neighbor-joining method with bootstrap analysis of 1000 replicates. In this study, the annual observation period for influenza viruses began in September and ended in August of the following year.

The results of genetic analysis of oseltamivir-resistant seasonal A(H1N1) and A(H1N1)pdm09 influenza viruses are presented in Table 1. A total of 30 out of 60

Table 1. Seasonal A(H1N1) and A(H1N1)pdm09 influenza viruses resistant to oseltamivir in Nara Prefecture

Season	Seasonal A(H1N1)			A(H1N1)pdm09		
	No. of viruses	No. of viruses tested	No. (%) of viruses with H275Y	No. of viruses	No. of viruses tested	No. (%) of viruses with H275Y
2007/2008	39	28	0 (0)	—	—	—
2008/2009	53	32	30 (93.8)	240	43	0 (0)
2009/2010	—	—	—	414	74	3 (4.1)
2010/2011	—	—	—	240	180	5 (2.8)
2011/2012	—	—	—	—	—	—
2012/2013	—	—	—	8	8	0 (0)
Total	92	60	30 (50.0)	902	305	8 (2.6)

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Table 2. Inhibition of the enzyme activity of A(H1N1)pdm09 influenza viruses isolated in Nara Prefecture

Virus	Amino acid at position 275 in the NA gene	IC ₅₀ values in the NA inhibition assay (nM)			
		Oseltamivir	Peramivir	Zanamivir	Laninamivir
A/NARA/2/2010	Histidine	0.11	0.06	0.25	—
A/NARA/39/2010	Tyrosine	32.92	13.45	0.21	—
A/NARA/42/2010	Histidine	0.07	0.06	0.23	—
A/NARA/43/2010	Histidine	0.07	0.05	0.21	—
A/NARA/44/2010	Histidine	0.07	0.05	0.26	—
A/NARA/89/2010	Histidine	0.29	0.12	0.34	0.32
A/NARA/48/2011 ¹⁾	Histidine	0.48	0.12	0.69	0.60
A/NARA/54/2011 ¹⁾	Tyrosine	101.99	12.36	0.49	0.66
A/NARA/56/2011 ¹⁾	Tyrosine	132.34	16.69	0.52	0.60
A/NARA/59/2011 ¹⁾	Tyrosine	123.64	15.21	0.50	0.58

¹⁾: isolated from the hematology ward patients.

(50.0%) seasonal A(H1N1) viruses possessed a point mutation at the amino acid position 275 in the NA gene. The H275Y mutation in the NA gene was identified in 93.8% of the seasonal A(H1N1) viruses that were circulating during the 2008/2009 season. In addition, eight out of 305 (2.6%) A(H1N1)pdm09 viruses possessed a point mutation at the amino acid position 275 in the NA gene. The seasonal ratio of A(H1N1)pdm09 viruses with the H275Y mutation were 0% and 4.1% during the 2008/2009 and 2012/2013 seasons, respectively. The IC₅₀ values of A(H1N1)pdm09 viruses with the H275Y substitution to NA inhibitors are shown in Table 2. The H275Y mutant viruses showed approximately 200- and 100-fold increases in the IC₅₀ values to oseltamivir and peramivir, respectively, when compared with those of a 2011/2012 isolate without the H275Y mutation. However, the H275Y mutation had no impact on the susceptibility of the virus to zanamivir or laninamivir. Nine A(H1N1)pdm09 viruses, including three resistant virus isolates from the 2009/2010 to 2010/2011 seasons, were used for phylogenetic analysis (Fig. 1). The three resistant viruses (NARA/54/2011, NARA/56/2011, and NARA/59/2011) with the H275Y mutation possessed the same HA and NA amino acid sequences (accession no.: NARA/59/2011: EPI321490 [HA sequence] and EPI321489 [NA sequence]).

Japan presents the highest per capita usage of oseltamivir in the world (1); hence, the emergence and spread of oseltamivir-resistant viruses are a matter of concern (3,6). It has been reported that A(H1N1) viruses develop resistance to oseltamivir through a single amino acid substitution at the position 275 in the NA gene (13). During the 2008/2009 season, seasonal A(H1N1) influenza viruses with the H275Y mutation were widespread throughout Japan (3,4), and our results showed that these viruses caused an epidemic in Nara in the 2008/2009 season. In the present study, the total frequency of oseltamivir-resistant A(H1N1)pdm09 viruses was 2.6% (8/305) from the 2008/2009 to 2012/2013 season. These results suggest that oseltamivir-resistant A(H1N1)pdm09 viruses did not spread in Nara Prefecture. On the other hand, the rapid emergence of oseltamivir-resistant A(H1N1)pdm09 viruses has been reported in hospitalized patients (14). In the United Kingdom, Moore et al. described the evidence of human-to-human transmission of oseltamivir-resistant

A(H1N1)pdm09 viruses in a hematology unit (15). Furthermore, in Nara Prefecture, oseltamivir-resistant A(H1N1)pdm09 viruses were detected in the hematology ward during the 2010/2011 season (16). This case was the second cluster of A(H1N1)pdm09 viruses with the H275Y mutation with suspected human-to-human transmission in Japan (7). Four oseltamivir-resistant A(H1N1)pdm09 viruses occurred in this case (three viruses were detected in patients, and surprisingly, one was later detected in a nurse). The results of the NA inhibition assay show that the H275Y mutant viruses exhibit resistance not only to oseltamivir but also to peramivir. The phylogenetic analysis of the NA and HA1 genes show that three H275Y mutant viruses belong to the same cluster in the NA and HA1 phylogenetic tree. These three viruses possess the same HA and NA amino acid sequences. Moreover, a mutant virus was detected in a nurse, which suggests the possibility of human-to-human transmission of this virus.

Among the eight oseltamivir-resistant A(H1N1)pdm09 cases, five were associated with treatment, two were associated with post-exposure prophylaxis, and one had an unknown association. To our knowledge, these eight oseltamivir-resistant A(H1N1)pdm09 viruses did not spread to the community.

Since the 2011/2012 season, A(H1N1)pdm09 virus has not become predominant in the influenza season in Japan. However, two A(H1N1)pdm09 virus outbreaks occurred in Nara Prefecture early in the 2012/2013 season. Fortunately, no oseltamivir-resistant A(H1N1)pdm09 virus was detected in either case. The continuous monitoring of A(H1N1)pdm09 viruses with the H275Y mutation is important as an effective antiviral treatment strategy not only at the national level but also at the community level.

Our results showed a high frequency of oseltamivir-resistant seasonal A(H1N1) viruses in the 2008/2009 season and a low frequency of oseltamivir-resistant A(H1N1)pdm09 viruses during the 2008/2009–2012/2013 season in Nara Prefecture, although only a small number of viruses had been tested. A cluster of oseltamivir-resistant A(H1N1)pdm09 virus infection in the hematology ward suggests the possibility of human-to-human transmission of the resistant viruses. Because oseltamivir-resistant seasonal A(H1N1) viruses rapidly circulated in the 2008/2009 season, regional studies

A

0.001

B

0.001

Fig. 1. Phylogenetic trees of the A(H1N1)pdm09 virus HA1 (A) and NA (B) genes. Phylogenetic trees of HA1 (A) and NA (B) genes were constructed using the neighbor-joining method with bootstrap values of 1,000 replicates. Amino acid substitutions relative to the A/California/07/2009 virus are shown on the left side of the node. Black circles represent the H275Y mutant viruses and the 275H wild-type viruses are represented by white circles. The scale bar shows nucleotide substitutions per site.

must be conducted to monitor oseltamivir-resistant A(H1N1)pdm09 viruses at the community level in Japan.

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Conflict of interest None to declare.

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