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High Incidence of Amantadine-Resistant Influenza H1N1 Viruses Isolated during the 2007-2008 Season in Nara Prefecture, Japan

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Amantadine is the first anti-influenza drug licensed in Japan. The drug inhibits virus replication during the early stage of infection by blocking the ion channel formed by the M2 protein. A single substitution at 1 of 4 residues of M2 protein (positions 26, 27, 30 and 31) has been shown to confer resistance to adamantane. In 2005, Bright and colleagues’ genotypic study showed a great increase in the prevalence of drug-resistant H3N2 strains in southeastern Asian countries such as the mainland of China and Hong Kong (1). An extensive increase of amantadine-resistant influenza H3N2 viruses was also reported in Asia, Australia, New Zealand and North America (2-4). In many cases, the predominant amino-acid substitution occurred at position 31 (AGT → AAT: Ser to Asn) in H3N2 strains. In contrast, the prevalence of resistant H1N1 virus was not found until the 2005-2006 season. In this study, we have investigated 33 influenza H1N1 viruses isolated during the 2007-2008 season in Nara Prefecture for the possible emergence of the drug resistance to amantadine.

Throat swab specimens were collected from 41 children (mean age, 7.2 years old). Supernatants of throat swabs were inoculated into MDCK cells for influenza virus isolation. Finally, 33 influenza H1N1 strains were obtained. Viral RNA was extracted using the QuickGene SP kit (Fuji Photo Film, Tokyo, Japan) according to the manufacturer’s instructions, and then reverse transcription was performed to create complementary DNA using M2-Rev primer. PCR was performed using specific primers (M2-For3 and M2-Rev) to amplify the M2 coding region in segment 7, as described previously (5). In addition, the PCR products were partially sequenced using a sequencing kit (Thermo Sequenase Cy5.5 Dye Terminator Cycle Sequencing Kit; GE Healthcare UK Ltd., Buckinghamshire, UK). Further, a BLAST search was performed against GenBank to analyze DNA homology. The results of the analysis of amantadine-resistant influenza H1N1 viruses, including the mutation site and monthly prevalence, are summarized in Table 1. Twenty out of 33 (60.6%) strains had an amino acid change from Ser to Asn at residue 31 (AGT → AAT). This is the most common mutation known to confer resistance to amantadine. The incidence of resistant viruses was elevated in the period of the influenza season, while in October and November, there was no resistant virus detected. However, in December, 8 viruses out of 12 (66.6%) were resistant; in January, 6 out of 11 (54.5%) were resistant; and in February and March, 3 out of 3 (100%) and 3 out of 3 (100%) were resistant, respectively. The results of a BLAST search revealed that the 20 resistant strains shared high homology with A/Colorado/UR06-0053/2007 and A/Arizona/1/2006 (GenBank accession no. CY026524 and EU100612, respectively).

We report here a high incidence of amantadine resistance

Table 1. Incidence of amantadine-resistant influenza H1N1 viruses in 2007-2008 season in Nara Prefecture, Japan

<table>
<thead>
<tr>
<th>Month</th>
<th>No. of specimens isolated</th>
<th>No. of strains with amino acid substitution (%)</th>
<th>No. of strains with amino acid substitutions in the M2 gene&lt;sup&gt;1)&lt;/sup&gt; at position</th>
</tr>
</thead>
<tbody>
<tr>
<td>October</td>
<td>1</td>
<td>0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>November</td>
<td>3</td>
<td>0</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>December</td>
<td>12</td>
<td>8 (66.6)</td>
<td>0 0 0 8</td>
</tr>
<tr>
<td>January</td>
<td>11</td>
<td>6 (54.5)</td>
<td>0 0 0 6</td>
</tr>
<tr>
<td>February</td>
<td>3</td>
<td>3 (100.0)</td>
<td>0 0 0 3</td>
</tr>
<tr>
<td>March</td>
<td>3</td>
<td>3 (100.0)</td>
<td>0 0 0 3</td>
</tr>
<tr>
<td>2007-2008 season</td>
<td>33</td>
<td>20 (60.6)</td>
<td>20</td>
</tr>
</tbody>
</table>

<sup>1)</sup> Substitution position of the amino acid in the M2 protein.

<sup>2)</sup> AGT → AAT (Ser → Asn).
among influenza H1N1 viruses during the 2007-2008 influenza season in Nara Prefecture. The majority of drug-resistant H1N1 viruses, similar to resistant H3N2 viruses, contained a mutation at position 31. Though all were transition mutations, there may be some unknown mechanisms for the induction of mutations.

Amantadine has been used for many years because of its wide availability and low cost, yet the frequency of amantadine resistance among field isolates has been low (<1%) until recently. Actually, based on worldwide epidemiological surveillance data, Ziegler et al. reported a low frequency (0.8%: 16/2,017) of resistant viruses among H1N1 and H3N2 viruses during a 4-year period of time (4). However, the situation has changed dramatically. Deyde et al. reported a comprehensive analysis of influenza H3N2 and H1N1 viruses isolated globally in 2004-05 and 2005-06 (5). According to the report, the frequency of resistant H1N1 viruses increased to 71.7% (33/46) for the entire 2005-2006 season in China. Similarly, 44.8, 23.5 and 33.3% of the viruses obtained from Europe, Taiwan and Canada, respectively, were drug resistant. In Japan, the proportion of amantadine-resistant H1N1 isolated was 0% (0/2) in 2005-06 (5). Besides influenza A(H3N2) and A(H1N1) viruses, highly pathogenic avian influenza A viruses of the H5N1 subtype are circulating in eastern Asia with unprecedented epizootic and epidemic effects. Cheung et al. reported the distribution of genetic mutations associated with resistance to the M2 protein among H5N1 virus isolates in Vietnam, Thailand, Cambodia and Indonesia (6). More than 95% of the viruses isolated in Vietnam (162/175) and Thailand (58/58) contained a mutation that causes resistance. Recently, Mase et al. reported that the amantadine-resistant H9N2 viruses were isolated from chicken products imported from China (7).

In the interest of public health, it is essential to monitor drug resistance systematically, and to evaluate the clinical efficacy of antiviral treatment in patients infected by drug-resistant viruses.

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REFERENCES